सत्यमेव जयते

INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI

I.A.R I.6.
GIP NLK—H 3 I.A.R.I. 10 5 55—15,000

JOURNAL
OF
DAIRY SCIENCE

VOLUME XIV

JANUARY, 1931, to NOVEMBER, 1931

1931
THE WILLIAMS & WILKINS COMPANY
BALTIMORE, U. S. A.

EDITORIAL BOARD

A. C. DAHLBERG, *Editor*
Geneva, N. Y.

ASSOCIATE EDITORS

C. H. Eckles
St. Paul, Minn.
E. G. Hastings
Madison, Wis.
O. F. Hunziker
Chicago, Ill.

C. W. Larson
Buffalo, N. Y.
E. B. Meigs
Baltimore, Md.
L. A. Rogers
Washington, D. C.

H. A. Ruehe
Urbana, Ill.
Paul F. Sharp
Ithaca, N. Y.
C. E. Gray
San Francisco, Calif.

All correspondence regarding articles should be directed to A C Dahlberg, Editor, Geneva, N. Y

All correspondence regarding advertising should be directed to the Publishers, The Williams & Wilkins Company, Baltimore, Md.

CONTENTS

JANUARY. No. 1

The Effect of the Hydrogen Ion Concentration on the Churning Time of Cream. E. S. Guthrie and Paul Francis Sharp 1

Size of Cow and Efficiency of Milk Production. W. L. Gaines... 14

Bromcresol Green Paper in the Manufacture of Grain-Curd Casein. E. O. Whittier 26

Observations on the Heat Resistance of Some Ropy Milk Organisms. B. W. Hammer and R. V. Hussong 27

Variations in the Coagulation and Proteolysis of Milk by Streptococcus lactis. L. A. Harriman and B. W. Hammer 40

Variations in the Buffer Value of Herd Milk. Paul D. Watson . .... , 50

Viability of Lactobacillus acidophilus and Lactobacillus bulgaricus Cultures stored at Various Temperatures. Luther A. Black 59

Correlation Between Annual Butter Fat Production and Annual Feed Cost of Dairy Cows under Farm Conditions. C. W. McIntyre.... 73

Some Observations on the Basic Viscosity of Ice Cream Mixes. J. C. Hening. 84

MARCH. No. 2

A Test for the Detection of Milk Unstable to Heat. Guy A. Ramsdell, Wm. T. Johnson, Jr., and Fred R. Evans 93

Speed of Dasher and Scraper as Affecting the Quality of Ice Cream and Sherbet. E. L. Reichart 107

An Interpretation of the Feeding Standards for Growing Dairy Cattle. J. B. Fitch and R. H. Lush........ 116

The Effect of Feeding Menhaden (Fish) Oil on the Secretion of Milk and the Composition of Butter Fat in the Dairy Cow. J. B. Brown and T. S. Sutton. 125

The Effect of Dilution on the Titratable Acidity of Cows' Milk. H. H. Sommer and Jules Ménos... 136

Agglutinin, Complement, and Chemical Composition of the Colostrum of the Cow. M. Sato, K. Ogura and H. Ikejima.. 156

Calculating Rations for Dairy Cows. T. E. Woodward 173

The Feathering of Evaporated Milk in Hot Coffee. Randall Whitaker. .. 177

MAY. No. 3

Skimmilk as a Feed for Dairy Cows. C. H. Eckles and E. N. Shultz 189

The Effect of Acidity on Lactobacillus acidophilus Cultures. Luther A. Black and J. C. Harris......................... 198

The Number of Daughters Necessary to Prove a Sire. Jay L. Lush.. 209

The Influence of Sweetened, Frozen Cream on the Development of Swell in Ice Cream. Walter V. Price.... 221

Fat Soluble Vitamins. XXXIII. The Determination of Vitamin A and Its Stability in Butter Fat to Ultra Violet Radiations. H. Steenbock and Alice M. Wirick 229
The Relation of Certain Plant Processes to Flavor Development in Market Milk. P. H. Tracy and H. A. Ruehe 250
Calcium and Phosphorus Metabolism in Dairy Cows. IV. The Assimilation of Calcium Fed as Calcium Gluconate. William A. Turner, Edward A. Kane, Walter S. Hale and Herbert G. Wiseman 268
The Germicidal Action of Raw Milk upon an Organism of the Sweet Curdling Type. Harold R. Curran 276

JULY. No. 4

Deficiencies in Rations Devoid of Roughage for Calves. I. The Effect of the Addition of Cod Liver Oil and Alfalfa Ash. S. W. Mead and W. M. Regan. 283
Length of Calving Interval and Average Milk Yield. W. L. Gaines and J. R. Palfrey 294
Dietary Factors Influencing Calcium Assimilation. XIV. The Influence of Mineral Acids and Sugar on the Calcium Metabolism of Milking Cows. E. B. Hart, H. Steenbock and O. L. Kline 307
Calcium Assimilation as Indicated by Bone Analysis in Long-Time Experiments. Arthur M. Hartman and Edward B. Meigs 322
Properties of Some Acid Caseins. R. W. Bell and S. P. Gould 337
Method for Hydrogen Ion Determination of Butter. O. F. Hunziker, W. A. Cordes, and B. H. Nissen 347
The Hemoglobin and Total Phosphorus in the Blood of Cows and Bulls. C. M. McCay 373

SEPTEMBER. No. 5

The Contribution of the Dam in Inheritance of Milk and Butterfat. Lynn Copeland 379
Uniform Rules for the Herd Test 394
The Churn as a Source of Molds in Butter. H. Macy, W. B. Combs and H. B. Morrison, Jr. 398
Preliminary Studies of Churn Sanitation. H. B. Morrison, Jr., H. Macy and W. B. Combs 404
The Solubility of Metals in Milk. I. The Solubility of Copper under Various Conditions. H. T. Gebhardt and H. H. Sommer 416
The Relative Antineuritic (Vitamin "B") Potency of Certain Milk Derivatives. G. C. Supplee, O. J. Kahlenberg, G. E. Flanigan and O. D. Dow .. 447
Malt Flavor in Raw Milk Produced by Micrococcus. P. H. Tracy and R. J. Ramsey 457
A Study on Methods for Determining Numbers of Moulds and Yeasts in Butter. I. The Relation of the pH of the Medium. A. H. White and E. G. Hood 463

NOVEMBER. No. 6

Further Remarks on Data on Bacterial Counts Presented by Brew and Dotterrer and Analyzed by Brew. G. A. Baker 477

The Influence of Environmental Temperature on the Percentage of Butter Fat in Cow's Milk. H. J. Brooks. 483
A Study of Methods for Determining Numbers of Moulds and Yeasts in Butter. 2. The Influence of Temperature and Time of Incubation. A. H. White and E. G. Hood 494
Effect of Single and Double Homogenization of Cream upon Coagulation by Heat and Rennet and upon Separation of the Fat. B. H. Webb. 508
The Relation of Feathering and Heat Stability of Cream to Fat Clumping Produced by Homogenization F. J. Doan. 527
Proceedings of the Twenty-Sixth Annual Meeting of the American Dairy Science Association. 540
Index. 551

THE EFFECT OF THE HYDROGEN ION CONCENTRATION ON THE CHURNING TIME OF CREAM*

E. S. GUTHRIE AND PAUL FRANCIS SHARP

Department of Dairy Industry, Cornell University, Ithaca, N. Y.

INTRODUCTION

Those familiar with churning generally recognize that as the acidity in cream develops, the time required for churning decreases. This increase in acidity, due to natural souring, covers only a rather narrow range of pH; namely, from about 6.8 to 4.4. It is probable that in certain cases cream may be neutralized, so that churnings might be carried out at a pH slightly above 7. As long ago as 1879, Clausnizer (2) reported that cream from milk to which considerable amounts of alkali had been added, could be churned; Babcock (1) confirmed this finding.

Ruehe and Stiritz (3) used the fat loss in the buttermilk as an index of various factors affecting the stability of cream. Their experiments were with neutralized cream. They found that the addition of 1 per cent of sodium chloride and of hydrochloric acid in an amount sufficient to increase the acidity by 0.05 per cent expressed as lactic acid, decreased the fat lost in the buttermilk. A combination of both sodium chloride and hydrochloric acid was most effective.

Van Dam and Holwerda (4) diluted ripened cream with sour milk plasma and milk serum in such proportions that increasing amounts of casein resulted. In one series the creams ranged in pH from 4.47 to 4.50. The churning time at first decreased slightly with an increase in casein, and then increased again with the larger amounts of casein. The fat loss in the buttermilk decreased uniformly as the amount of casein increased. They (5) also studied the effect of decreasing the concentration of the water soluble constituents by diluting cream with either milk

*** Presented at the Madison, Wisconsin, Meeting of the American Dairy Science Association June 27, 1928. Received for publication May 19, 1930.**

serum or water, and found that the dilution of the water soluble constituents increased churning time slightly and decreased the fat loss in the buttermilk. Van Dam and Holwerda (6) studied the influence of acidity as caused by acid developed by ripening. Although the difference in acidity amounted to only a few tenths of a pH (in one series from an average of 4.59 to 4.39), yet a decrease in fat loss was noted from 0.52 to 0.42. Little influence was exerted on the churning time.

Many other investigators have carried out experiments in which the influence of starter and development of acidity were studied. So fa. as we were able to discover, no one had studied the effect of pH on churning over a wide range. Such a study was, therefore, undertaken in the hope that some light might be thrown on the mechanism of churning. It was believed that the stability of the cream or the resistance of cream to churning would be most accurately evidenced by the churning time. The effect of pH on the churning time, therefore, was studied.

EXPERIMENTAL

Description of apparatus

In order to carry out such an investigation, it was necessary to control the other factors, besides the pH. Very few churnings have been executed under, what would be considered, accurately controlled experimental conditions. Since cream is usually changing in its churning properties, the carrying out of a series of churning experiments, one after the other in the same churn, may not give results which are exactly comparable with each other, due to such factors as the change in acidity, aging of the cream, and change in the proportion of liquid and solid fat in the fat globules. In addition, the temperature of the cream before and during the churning process may not be adequately controlled.

In our attempt to perform controlled churning experiments, 4 one-gallon glass churns containing agitators were so arranged that the glass parts of the churns were almost completely immersed in a water thermostat which was vigorously stirred. These 4 churns were all driven from the same line shaft by a relatively powerful electric motor. The driving mechanism was composed entirely of gears so that there was no chance for one churn to run more slowly than the others or the whole set to run more slowly one time than another. The outfit as shown in figure 1, was so arranged that a churn could be removed or started at will without stopping any of the others. The motor was powerful enough so that the removal or addition of the churns produced no appreciable effect upon its speed. In all of the work reported here the churnings were carried out in sets of four,

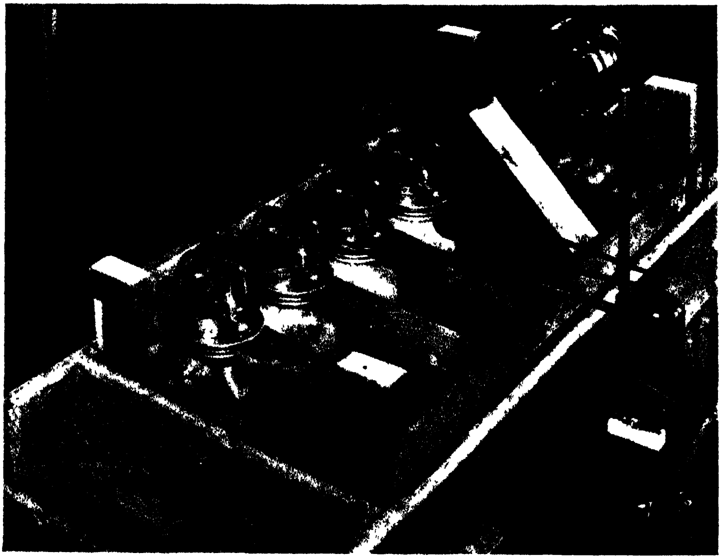


FIG 1 APPARATUS FOR EXPERIMENTAL CHURNING INVESTIGATIONS

and in every case one churn was operated as a control as a basis for comparison of the variable factors which were being studied in the other three. Before the motor was started, the cream was placed in the churns and the churns were set in the water bath for one hour to permit the cream to come to the churning temperature. The cream had previously been held at 2°C. for at least twelve hours. The water thermostat was kept at 17°C. (62.6°F.)

The procedure which was used in adjusting the cream to the various hydrogen ion concentrations was as follows: Cream from fresh milk was separated, standardized to 40 per cent fat, and pasteurized. To 1125 gms. of this sweet cream were added 375 cc. of water or solution, giving as a result cream of 30 per cent fat content. In the case of the control churning, 375 cc. of distilled water were added. To the other three churns 375 cc. of a solution, which contained the appropriate amount of hydrochloric acid, sodium hydroxide, or acid or alkali plus salt to give the pH desired, were added.

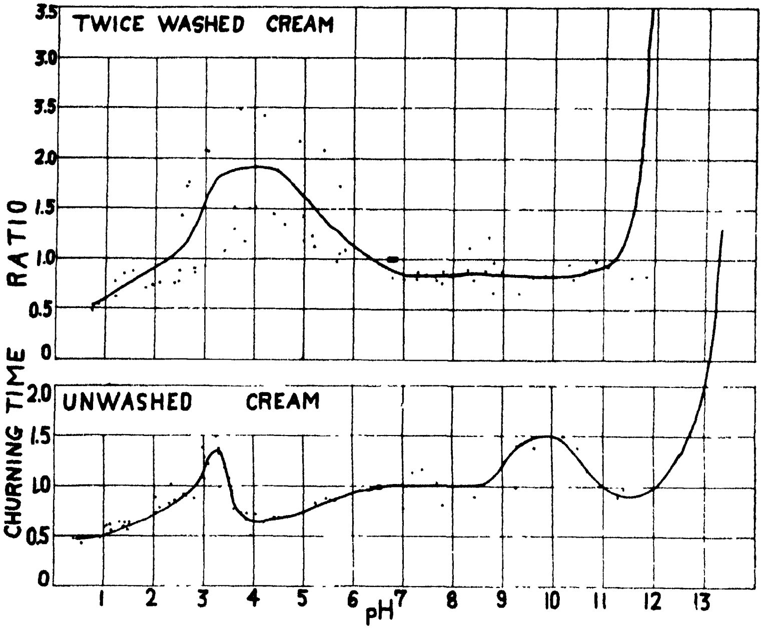


FIG 2 RELATION BETWEEN pH AND CHURNING TIME OF WASHED AND UNWASHED CREAM

Churning time of the control taken as 1

It was found that, even with the precautions taken and the similarity in the churns, the four churns did not churn the control cream (1125 grams of sweet cream plus 375 cc. of distilled water) in the same time, but that some of the churns consistently required more time to churn the same cream than did other churns. Several churnings were made, in which the churns were all filled with the same cream, and from the average results so obtained, ratio-time factors were calculated so that this difference in time required to churn the control sample could be corrected for when the variously treated creams were run in the same churns. One churn was always operated with the control cream.

Churning washed and unwashed cream at various pH values

The results obtained on adjusting the unwashed cream to various hydrogen ion concentrations are shown in figure 2 in which the results are expressed on the basis of the churning time of the control as 1.0. the average pH of the control was about 6.6 to 6.7. The usual churning time of the control was 20 minutes. As the acidity was increased, the churning time decreased until a minimum was reached at about pH 4.2. As the hydrogen ion concentration was increased still further, the churning time increased until a maximum was reached at a pH of a little above 3. From there on the churning time steadily decreased as the hydrogen ion concentration increased. On the addition of alkali to the cream, the churning time at first remained constant and then increased to a maximum at near pH 10. As still more alkali was added, the churning time decreased to a minimum at near pH 11.5, and upon further additions of alkali, the churning time increased fairly rapidly until the curve evidently approached an asymptote of infinite churning time at somewhere near pH 13.5.

This curve brings out at least two interesting points. The first is the fact that churning can be obtained over such an extended range of pH; the second is the apparent relationship of long churning times to dispersion or hydration of the casein and of the short churning times to its precipitation or dehydration. The isoelectric point of casein is near a pH of 4.7, in which region the casein is not in solution and the churning time is at a minimum. As pH 3.0 is approached, the casein is dispersed and the churning time is lengthened. As pH 1 is approached casein again is precipitated and the churning time becomes shorter.

Several investigators of the viscosity of alkaline casein solutions have found a maximum near pH 9 to 10 and our experiments show that in this region the churning time is lengthened. In the highly alkaline solutions, that is pH 12 to 13, we again find a great increase in the churning time which may perhaps be due to soap formation. The effect of alkali on the churning time at the high pH values shows a distinct difference from the effect of acid, at the very low pH values.

Still further information on the factors influencing the churning time was obtained by churning twice washed cream. The cream was first separated to give about 40 per cent fat. To this cream was then added 9 volumes of water and the cream again separated. To the cream so obtained 9 additional volumes of water were added and the separation was again carried out. The resulting cream was standardized to 40 per cent fat with water and is called in figure 2 the "twice washed cream." Such washing should, by straight dilution as calculated, reduce the plasma constituents in the free liquid phase to about 1/250 of their concentration in the unwashed cream. The actual reduction is, however, probably considerably less due to adsorption on the surface of the fat globules and the probable change in the amount adsorbed after each washing. The churnings and the adjustment of the pH were carried out in the same manner as described for unwashed cream. The churning time of the control was about thirteen minutes. The data are presented graphically in figure 2. It will be noted that the curves for the twice washed cream and the unwashed cream are distinctly different. In the case of the twice washed cream as the acidity is increased, there is a distinct increase in churning time up to a maximum in the neighborhood of pH 4.0. From there on as the hydrogen ion concentration increases there is a decrease in churning time. This maximum at pH about 4.0 for the twice washed cream is almost exactly at the same hydrogen ion concentration as the minimum for the unwashed cream. The two sets of points in the acid region where one set is above the curve and the other below are due to the use of two different lots of cream.

As alkali is added to the washed cream there is at first a slight decrease in churning time, followed by a horizontal portion which indicates that changing the pH through the region from 7 to 10.5 exerts no effect on the churning time. Upon increasing the pH above 11.0 the churning time increases tremendously, possibly approaching infinite churning time at some place near pH 12.5. The pH churning time curve for the unwashed cream is a fairly typical casein curve, while the pH churning time curve for the twice washed cream is distinctly not a casein curve. Since the importance of the casein as a factor in regulating the churning time is indicated by these two curves, other experiments were devised to show its influence.

Churning in the presence of $NaCl$ and $CaCl_2$ at various pH values

When water is made half normal with respect to sodium chloride, casein does not dissolve on the acid side of the isoelectric point, that is near pH 3. If the shortness of the churning time is correlated with separation of the casein from solution, then by making the water of the cream half normal with respect to sodium chloride, there should be no lengthening of the churning time of the unwashed cream at near pH 3.2. Such experiments were carried out and the results are expressed graphically in figure 3. It will be noted that in the neighborhood of pH 3, while there is a very slight bump in the curve, yet the churning time is considerably shorter than the control, indicating that in this case the insolubility of the casein is associated with a short churning time. Approximately the same results were obtained when the water was made tenth normal with respect to sodium chloride. In the cases where the pH of the creams containing sodium chloride were increased, maxima were obtained in the neighborhood of 9.5 and 10, which corresponds to the maximum obtained with cream to which no salt was added. Sodium chloride does not render the casein insoluble on the alkaline side of the isoelectric point. In the presence of the sodium chloride as the pH approached the high alkalinities, the churning time increased to infinity. The break in the curve toward infinity seemed to occur at a lower pH the higher the concentration of sodium chloride.

When the water of the cream was made half normal with

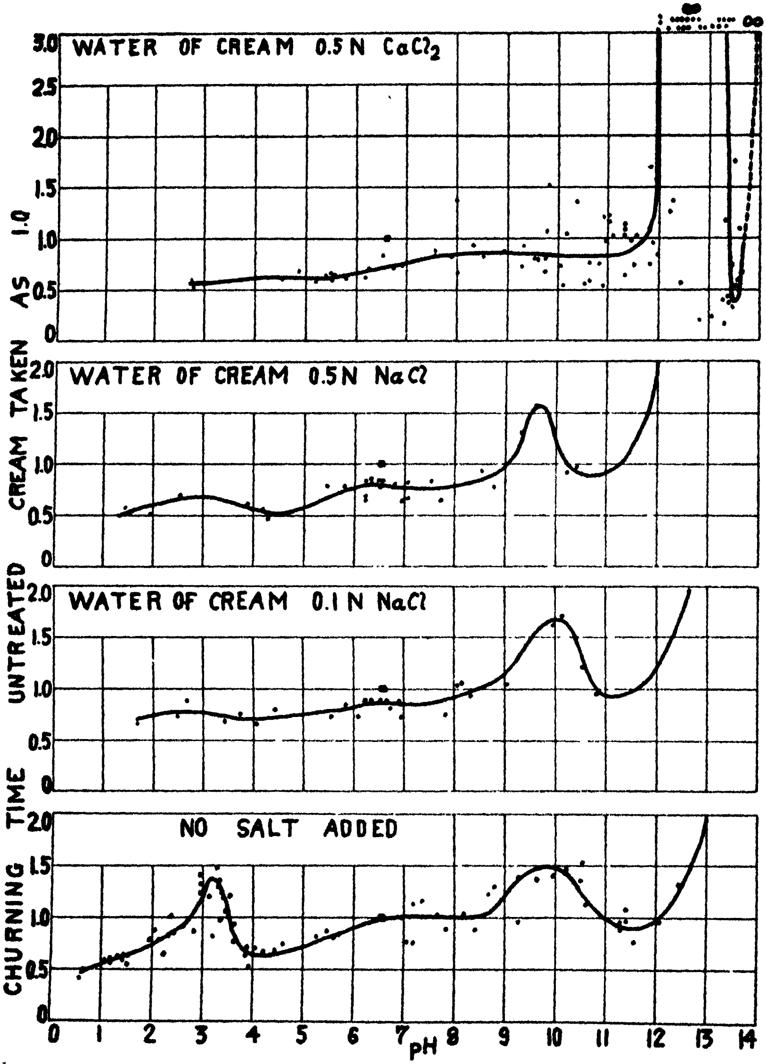


FIG. 3. RELATION BETWEEN pH AND CHURNING TIME OF CREAM TO WHICH SODIUM OR CALCIUM CHLORIDE HAD BEEN ADDED

Churning time of the control taken as 1

respect to calcium chloride on the acid side of the isoelectric point, the results were very similar to those obtained with sodium chloride. Calcium chloride prevents the casein from dispersing near pH 3. The calcium chloride also caused a shortening of the churning time in the region between pH 7.0 and 11.5. In the case of the addition of calcium chloride there was no hump in the curve at pH 10.

In the presence of calcium chloride the churning time approaches infinity (followed for 40 hours in some cases) between pH 12 and 13.3. Above this pH there is a narrow range where the churning time is very short and on the addition of still more alkali the churning time approaches infinity again. The actual pH values could not be determined in the presence of this high concentration of alkali or at least the readings were very erratic and indicated pH values between 8 and 10.

When considerable sodium hydroxide is added to calcium chloride a precipitate of the relatively insoluble calcium hydroxide is formed which results in the formation of considerable sodium chloride in the solution.

$$CaCl_2 + 2NaOH = Ca(OH)_2 + 2NaCl$$

Consequently some of the effects of the addition of sodium chloride might be indicated. The hump in the curve at pH 10 was not in evidence. The churning time did approach infinity at near pH 12 which corresponds to the rise in the curve with 0.5 normal sodium chloride.

The churning times at the high pH values are probably influenced by the hydrolysis of the fat and the formation of soaps. Since soaps tend to reduce the surface tension to a marked degree and alkalies tend to dissolve protein, it is conceivable at least that the material which is naturally adsorbed on the surface of the fat globules might be displaced by the sodium or calcium soaps. If a sodium soap was adsorbed, as might be expected in cases where the cream was simply made highly alkaline or was made alkaline in the presence of sodium chloride, one would expect an extremely stable emulsion, since sodium soaps form and stabilize emulsions of oil-in-water. One would hardly expect emulsions so stabilized to churn. Calcium soaps, on the other hand, tend to change emulsions of oil-in-water over to emulsions of water-in-oil; therefore, the presence of the calcium hydroxide in the highly alkaline solutions might tend to form calcium soaps and break the emulsion, forming butter. This is a possible explanation of the short churning times found at pH 13.5 when

TABLE 1

Effect of the degree of dispersion and hydration of the casein on the churning time

EXPERIMENT NUMBER	SUBSTANCE ADDED							CHURNING TIME
	Water	Skim-milk	Grain curd casein solution	Rennet casein suspension	Rennet extract	Sodium flouride	Hydrochloric acid	
1	+				−	−		1 00
	+				+	−		0 70
	+				−	+		1 04
	+				+	+		1 00
2		+			−	−		1 07
		+			+	−		0 51
		+			−	+		1 05
		+			+	+		0 90
3			+	−	−			1 04
			+	−	+			0 47
			−	+	−			0 75
			−	+	+			0 47
4	+	−					−	1 00
	−	+					−	1 07
	+	−					+	0 67
	+	−					+	0.71
5	+	−	−		−	−	−	1.00
	−	+	−		−	−	+	0 93
	−	−	+		−	+	−	1 17
	−	−	+		+	+	−	0 71
6	+			−	−	−		1.00
	+			+	−	−		0 86
	+			+	+	−		0 47
	+			+	+	+		0 70

calcium chloride was added. The reason for the infinite churning time at pH 12 to 13.3 in the presence of calcium chloride is not apparent.

Effect of degree of dispersion of the casein on churning time at pH 6.45 to 6.8

With the exception of the highly alkaline region, the churning time can be more or less correlated with the dispersion of hydration of the casein. In order to gain more information on the effect of casein on churning time, the degree of dispersion of the casein was changed by several combinations obtained by the addition of water, skimmilk, casein, rennet, sodium flouride, and acid. The results obtained are given in table 1.

Each experiment is the average of two or more sets of churnings. The churning time of the control, where only water was added, is given the value 1.00. All of the other churnings have been related either directly or indirectly to this value. The pH values all fell between 6.45 and 6.8, with the exception of the experiments in which hydrochloric acid was added where the pH was 4.4 to 4.6.

The additions listed in table 1 were made as follows: (1) water 375 cc., (2) skimmilk 375 cc., (3) grain curd casein—17 grams dispersed in 375 cc. of $CaCl_2$—NaOH solution to give pH 6.8, (4) rennet casein 17 grams finely ground suspended in 375 cc. of water, (5) rennet extract added casein allowed to coagulate, (6) sodium flouride to make the water of the cream 0.1 normal; in cases where rennet was also present, the rennet was allowed to act before the sodium flouride was added, and (7) enough hydrochloric acid to give a pH 4.4 to 4.6.

In some few cases the relative effects produced when one experiment is compared with another are not quantitatively comparable, yet table 1 does show some general relationship of these treatments. The addition of rennet, which brings the casein out of solution, causes a shortening of the churning time as does the addition of acid. The addition of sodium flouride which is a very effective peptizing agent especially for rennet casein or curd, tends to lengthen the churning time. The addition of the rennet casein suspension shortens the churning time, while the addition of skimmilk or a solution prepared from grain curd casein lengthens the churning time slightly. The churning time seems to be shortened most of all by the addition of milk or casein followed by the addition of rennet.

Quality of stored butter

The butter made in these various churnings was stored for seven months or longer at 0°F., and was then scored. The main results obtained on the relation of the various quality factors observed are given in figure 4. It will be noted that there are two regions in which the butter became white or bleached. Because a sample was bleached, it did not necessarily follow that it was also tallowy. The most acid samples were white, but were not tallowy. The butter had the best flavor between pH 6 and 9. Several defects made their appearance at pH 3 to 6, the samples being fishy, tallowy, and stale. Below pH 3 the samples tasted of acid and were tallowy down to pH about 1; from there on they did not taste tallowy. Above pH 9 the samples tasted soapy. It should be remembered that while the best flavor was found in the range from pH 6 to 9, the range of best flavor might be quite different if the cream had first been allowed to sour and had then been neutralized.

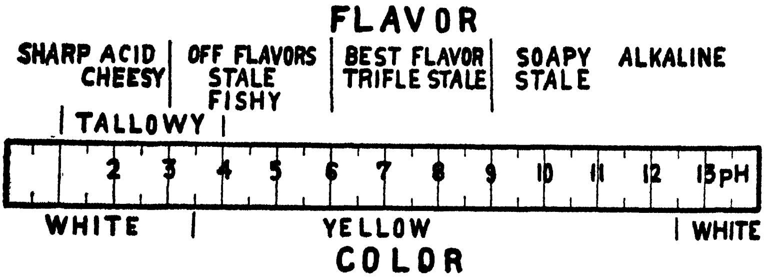


FIG. 4. RELATION BETWEEN pH OF THE CREAM AT THE TIME OF CHURNING TO THE QUALITY OF THE BUTTER AFTER STORAGE

SUMMARY

The effect of pH on churning time was investigated using cream, washed cream, and cream to which sodium and calcium chloride were added. The results obtained together with the effect of other means of increasing or decreasing the degree of dispersion or hydration of the casein, indicated that hydration or dispersion of the casein was associated with a lengthening of the churning time and that dehydration or precipitation was associated with a shortening of the churning time.

REFERENCES

(1) BABCOCK, S. M. 1889 The constitution of milk, and some of the conditions which affect the separation of cream. Wisc. Agr. Expt. Sta , Bul. No. 18.

(2) CLAUSNIZER, F. 1879 Ein nenes Aufrahmungs-Verfahren. Milch-Zeit., viii, 510–513.

(3) RUEHE, H. A , AND STIRITZ, B. A. 1925 Some studies in reducing the stability of butterfat emulsions. Jour. Dairy Sci , viii, 330–343.

(4) VAN DAM, W., AND HOLWERDA, B. J. 1927 De beteekenis van de gesuspendeerde kaasstof bij het verkarnen van zuren room. Verslagen Landbouwkundige Onderz. Rijkslandbouwproefstations, No. 32, p. 385–400.

(5) VAN DAM, W., AND HOLWERDA, B. J. 1927 De invloed van de concentratie der oplosbare eiwitstoffen van gezuurden room op het vetgehalte der karnemelk en op den karnduur. Verslagen Landbouwkundige Onderz. Rijkslandbouwproefstations, No. 32, p. 401–405.

(6) VAN DAM, W., AND HOLWERDA, B. J. 1927 Over den invloed van hooge zuurheidsgraden van den room op het vetgehalte der karnemelk en op den karnduur. Verslagen Landbouwkundige Onderz. Rijkslandbouwproefstations, No. 32, p. 406–410.

SIZE OF COW AND EFFICIENCY OF MILK PRODUCTION*

W. L. GAINES

Department of Dairy Husbandry, University of Illinois

When we speak of the efficiency of a machine, a process, or an animal, we commonly speak in terms of a ratio of one sort or another. Thus, Jordan (1) in speaking of the efficiency of various farm animals kept for food employs the ratio

$$\frac{\text{pounds of edible solids produced}}{\text{pounds of digestible organic matter consumed}}$$

For the case of the dairy cow the writer (2) has suggested a "coefficient of efficiency" similar in nature to Jordan's ratio. It is based on the known relation between the fat percentage of the milk and the energy or nutritive value of the milk, and, on the known feed requirements for lactation and body maintenance. The formula, based on a year period, is

$$CE = 52.6 \frac{FCM}{FCM + 8.847W}$$

where CE is the coefficient of efficiency, FCM[1] is 4 per cent milk in pounds, and W is live weight of the cow in pounds.

The formula is intended to express the percentage of the feed energy which is recovered as milk energy. The facts required for the estimate of the coefficient are the weight of the cow and the yearly yield of milk and fat. Clearly by the formula CE increases with yield and decreases with weight. It cannot be less than zero and approaches 52.6 as a limit. The limit, 52.6, represents the efficiency of the mammary gland as apart from the body. At a given live weight, say 1,000 pounds, the CE's cor-

---

* Received for publication June 12, 1930.

[1] $FCM = 0.4 \times \text{milk} + 15 \times \text{fat}$ all in the same unit of weight. One pound FCM = 340 calories. For review of the energy basis of measuring milk yield see Gaines (3).

responding to a few yields (pounds of 4 per cent milk per year) are as follows:

Yield	0	5,000	10,000	15,000	20,000
CE	0	19.5	27.9	33.0	36.5

It is well known that the higher the yield of milk the more economical the production per unit of milk. It is also well known that the larger the cow the higher the yield. These relations are perhaps responsible for the notion that large cows are more efficient than small cows.[2]

If efficiency is defined as above it is clear that the relative efficiency of the small and large cow will depend upon the relation between live weight and milk yield. McDowell (4) has recently published a summary of a large number of records from dairy herd improvement associations in the United States which shows the weight-yield relationship. It is proposed here to adapt his data to the efficiency formula given above.

McDOWELL'S DATA

The yield of 4 per cent milk and coefficient of efficiency have been calculated from McDowell's published figures and are given in table 1. The feed cost per hundred-weight of 4 per cent milk has been computed for purposes of comparison. For the sake of completeness the number of records is also given. Figure 1 shows graphically the computed data, omitting Shorthorns.

Study of table 1 and figure 1 shows that the 4 per cent milk or energy yield increases more or less regularly with size of cow in each of the breed classes. In the case of Guernseys and Ayrshires the yield curves for grade and registered cows are nearly coinci-

---

[2] A contributing cause is also to be found in the early work of farm management investigators who found in certain whole-milk districts that large cows were more profitable than small cows, and deduced a direct connection between size of cow and economy of production. This deduction overlooked the fact that (for example) the Holstein breed of large cows produces a low-energy milk and the Jersey breed of small cows produces a high-energy milk, while both milks sold at the same price. The large cow was more profitable not because of her size but because her milk sold at a higher price per calorie. This market advantage of low-energy milk is now pretty much a thing of the past in many sections of this country.

TABLE 1

Relation of size of cow to energy yield, calculated biological efficiency, and observed economic efficiency (derived from McDowell's data)

(Cows 5 to 9 years of age)

BREED	LINE*	COWS CLASSIFIED BY WEIGHT, POUNDS										
		600	700	800	900	1000	1100	1200	1300	1400	1500	1600
Grade Jersey	a	193	658	3,491	2,513	2,862	510	249				
	b	5,883	6,430	6,990	7,314	7,528	7,836	8,102				
	c	27.7	26.8	26.1	25.2	24.2	23.5	22.8				
	d	107	101	94	92	90	88	89				
Registered Jersey	a	38	197	1,629	1,222	1,479	235	76				
	b	6,060	6,847	7,257	7,594	7,858	8,023	8,220				
	c	28.0	27.6	26.6	25.7	24.7	23.8	23.0				
	d	111	107	99	97	98	102	100				
Grade Guernsey	a		234	2,091	3,107	7,179	2,252	1,371	149			
	b		6,399	6,825	7,110	7,428	7,679	7,992	7,877			
	c		26.7	25.8	24.8	24.0	23.2	22.6	21.4			
	d		103	91	89	88	89	88	85			
Registered Guernsey	a		19	311	502	1,487	568	341	54			
	b		6,454	6,817	7,161	7,278	7,534	7,723	7,707			
	c		26.8	25.8	24.9	23.7	23.0	22.2	21.1			
	d		105	109	105	106	105	104	109			
Grade Ayrshire	a			42	56	167	95	77	14			
	b			6,603	6,472	6,890	7,562	8,089	8,077			
	c			25.4	23.6	23.0	23.0	22.7	21.7			
	d			97	90	90	91	87	99			

Registered Ayrshire..........	a			25	39	216	113	77	8			
	b			6,199	6,077	6,942	7,649	7,612	9,195			
	c			24 6	22 8	23 1	23 1	22 0	23.4			
	d			108	118	102	102	106	87			
Grade Holstein.............	a			1,230	2,666	13,658	10,616	13,933	3,130	1,959	228	87
	b			6,649	7,030	7,437	7,834	8,191	8,629	8,941	9,267	9,128
	c			25 5	24 7	24 0	23 5	22 9	22 5	22 1	21 6	20 6
	d			93	88	86	87	88	87	88	86	87
Registered Holstein.........	a			89	238	2,061	2,798	6,098	2,560	2,607	644	299
	b			6,874	7,663	7,952	8,327	8,807	9,329	9,549	9,904	10,511
	c			25 9	25 8	24 9	24 3	23 9	23 6	22 9	22 5	22.3
	d			105	100	96	96	95	95	97	98	98
Grade Shorthorn...........	a			330	632	3,121	1,781	1,897	351	157		
	b			5,806	5,888	6,196	6,465	6,847	7,206	7,318		
	c			23 7	22 4	21 7	21.0	20 6	20 3	19.5		
	d			81	78	77	77	79	79	82		

* Line a, number of records averaged.

Line b, average energy yield per cow per year, pounds 4 per cent n

Line c, calculated coefficient of efficiency $= 52.6 \frac{FCM}{FCM + 8.847W}$.

Line d, observed feed cost per hundredweight 4 per cent milk, cen

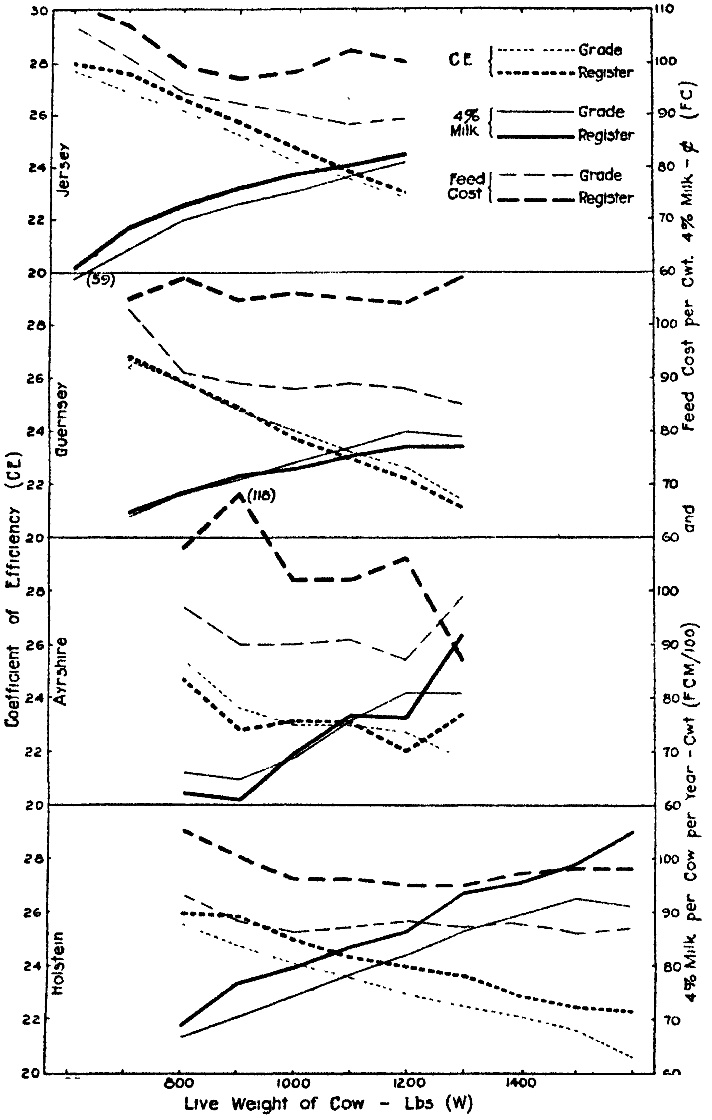


FIG. 1. RELATION OF SIZE OF COW TO ENERGY YIELD (4 PER CENT MILK), THEORETICAL EFFICIENCY, AND RECORDED FEED COST

dent. For Jerseys and Holsteins, on the other hand, the yield of the registered cows is consistently higher than that of grades of the same weight.

The figures for the calculated efficiency show a consistent trend, decreasing as weight increases. That is to say the increase in yield with increase in size is not sufficient to offset the extra feed requirements for maintenance. This holds true in all cases although in the Ayrshire breed the *CE* curve has little slope.

TABLE 2

Relation of size of cow to relative energy yield and efficiency

WEIGHT	NUMBER OF RECORDS	FCM 4 PER CENT MILK PER COW PER YEAR	FEED COST PER HUNDRED-WEIGHT 4 PER CENT MILK	CE $52.6 \frac{FCM}{FCM + 8.847W}$	CE′ $52.6 \frac{FCM}{FCM + 88.47W^{2/3}}$
pounds		*pounds*	*cents*		
600	231	6,284	104	28.5	26.3
700	1,108	6,874	99	27.7	26.1
800	9,238	7,236	92	26.6	25.6
900	10,975	7,416	90	25.4	24.9
1,000	32,230	7,539	89	24.2	24.2
1,100	18,968	7,736	90	23.3	23.7
1,200	24,119	8,016	90	22.6	23.4
1,300	6,266	8,359	90	22.1	23.3
1,400	4,723	8,548	91	21.5	22.9
1,500	872	8,773	91	20.9	22.7
1,600	386	9,136	91	20.6	22.6
All	109,116	7,737	90	23.6	24.0

The above table is derived from McDowell's data. The last line shows the average of all records as reported. The data for each breed class have been reduced by a factor such as to give an average for each breed class the same as the average for all records. The breed classes have then been thrown together by size classes, weighting by the number of records, which gives the averages shown in the body of the table.

Turning to the feed cost per hundred-weight of 4 per cent milk, pronounced trends are much less apparent. There is one striking and consistent feature about the feed-cost curves, namely, the feed cost per unit of milk energy is materially lower for grade cows than it is for registered cows. Even in the case of the Jerseys and Holsteins, where the yearly yield for registered stock is materially higher than that of the grade stock, it is nevertheless the case that the grades produce a unit of milk energy at lower feed cost. The difference amounts to about 10 cents per hundred-weight of 4 per cent milk. This is more than a 10 per cent advantage in feed cost in favor of the grade cows.

ALL BREED CLASSES COMBINED

In order to consider the above matters further the breed classes of table 1 have been combined and the results are given in table 2. The method of combining is outlined in connection with table 2, and was intended to regulate for the fact that the various breed classes appear in unequal numbers in the various weight classes. Figure 2 presents the combined data graphically.

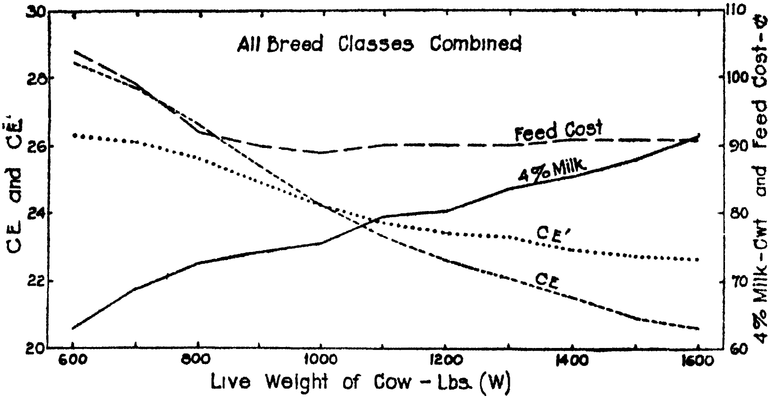


FIG. 2. COMPOSITE RELATION OF SIZE OF COW TO ENERGY YIELD (4 PER CENT MILK), THEORETICAL EFFICIENCY (*CE* AND *CE′*), AND FEED COST PER HUNDRED-WEIGHT OF 4 PER CENT MILK

All breed classes of table 1 combined

The yearly-yield curve, figure 2, becomes quite regular, is substantially linear, and has a slope corresponding approximately to an increase in yearly yield of 250 pounds of 4 per cent milk for each increase of 100 pounds in live weight of cow. It is of interest to note that in advanced registry records there is an increase in 365-day yield of about 500 pounds of 4 per cent milk for each increase of 100 pounds in live weight of cow (5), (6). Since the average of advanced registry records, ages 5 to 9 years, is also about double that of the present records it seems that the weight-yield relation is similar in the two classes of records, if the level of yield is taken as a base.[3]

A DISCREPANCY IN RESULTS

The feed cost per hundred-weight of 4 per cent milk as shown in figure 2 decreases rapidly over the size-of-cow range from 600 to 800 pounds. From 800 to 1,600 pounds live weight, feed cost per unit of milk is practically constant.

On the other hand, the coefficient-of-efficiency curve, figure 2, shows a continual decrease with size of cow. The feed cost curve is not consistent with the *CE* curve. The coefficient of efficiency represents the percentage of feed energy recovered as milk energy. Feed energy per unit of 4 per cent milk is therefore inversely proportional to *CE*. Ordinarily we should expect feed cost to be directly proportional to feed energy and accordingly the feed cost curve of figure 2 should be the inverse of the *CE* curve. If we plot the reciprocals of *CE*, table 2, the curve is fairly linear. If we further take the average relation of table 2 in which *CE* = 23.6 corresponds to a feed cost of $0.90 and apply it to the

---

[3] McDowell states: "This study showed that the same relationship in regard to production . . . as existed among individuals of the same breed did not exist among individuals of different breeds." Table 1 has been arranged to list the breeds in order of increasing average size. Diagonal reading of the yields in table 1 should therefore bear on the point. For greater convenience the following tabulation is repeated from table 1. It shows the effect of successive increases of 100 pounds in live weight on the yield of 4 per cent milk in hundredweights: (a) within the breed, from left to right; (b) breed to breed, from top to bottom of each column.

Grade Jersey	59	64	70	73	75	78
Grade Guernsey	64	68	71	74	77	80
Grade Ayrshire	66	65	69	76	81	81
Grade Holstein	70	74	78	82	86	89

Vertical reading, column by column, does not seem to support the quoted statement. In fact, the breed to breed comparison shows the increase in energy yield for each increase of 100 pounds in live weight to be very similar to that observed for all breed classes combined.

smoothed reciprocals we arrive at a feed cost of $0.75 per hundred-weight of 4 per cent milk at 600 pounds live weight, and $1.05 at 1600 pounds live weight. Each 100 pounds increase in weight should carry with it an increase in feed cost of 3 cents per hundred-weight of 4 per cent milk.

How is the discrepancy in the *CE* and feed cost curves to be accounted for?

Perhaps the *CE* formula is erroneous. If so then our feeding standards for dairy cows are also erroneous. We may consider the possibility that the body maintenance requirements of the cow vary as the 2/3 power of weight instead of being directly proportional to weight. Using the same maintenance requirement at 1,000 pounds live weight we have only to substitute $88.47W^{2/3}$ for $8.847W$ to obtain the new formula, which may be designated *CE'*.

The curve of *CE'* is given in figure 2 for comparison. It does not eliminate the inconsistency with the feed cost curve. It is probable that the actual nutritive requirements lie somewhere between that represented by the two curves, *CE* and *CE'*.

Perhaps the feed cost figures are erroneous, or perhaps feed cost is not proportional to feed energy. The work of Ross et al. (7) bears indirectly on the latter possibility and suggests that the ratio between feed cost and feed energy for marketable feeds would not be affected by size of cow.

It is possible that there may be systematic errors in estimating the feed consumed as between large and small cows, with a tendency to over-charge the small cow and under-charge the large cow. Particularly in the case of pasture this possibility is apparent, since there is a tendency to make the same pasture charge per month per cow regardless of the size of cow. The actual feed energy secured from pasture cannot be accurately estimated but it seems likely that the large cow may profit at the expense of the small cow as a consequence of the system of bookkeeping.

THE POINT OF VIEW

The above treatment of McDowell's data is one way of looking at the matter. McDowell (4) presents the material in quite a different way, primarily from the point of view of dollar returns per cow per year above feed cost. Of necessity there is a large margin between feed cost and value of product to allow for other costs and a profit. When this margin is multiplied by the amount of product it of course shows favorably to the large cow because of her larger production. It by no means establishes that the large cow is more efficient or even more profitable from a real net profit standpoint.

There can hardly be any doubt that, considering the cow as a machine we want a machine of some size.[4] But it seems to the writer that returns above feed cost may be a misleading way of comparison and lead to an exaggerated conception of the importance of size as one of the factors in the economics of milk production.

SUMMARY

McDowell's (4) published figures of 109,116 yearly records of cows 5 to 9 years of age in dairy herd improvement associations in the United States have been reworked to show, by live weight of cow and breeds: the energy yield in pounds of 4 per cent milk (340 calories); the theoretical coefficient of efficiency, that is, the percentage of feed energy recovered as milk energy; the recorded feed cost in dollars per hundred-weight of 4 per cent milk.

As between grade and registered cows energy yield is substantially equal for the Guernsey and Ayrshire breeds; but materially greater for the registered cows in the case of the Jersey and Holstein breeds.

In all 4 breeds the feed cost per hundred-weight of 4 per cent milk is lower for the grade cows, to the extent of about $0.10. The average feed cost is $0.90 per hundred-weight of 4 per cent milk for all records, the average yearly yield being 7,737 pounds of 4 per cent milk.

---

[4] For example, the goat is said to be more efficient than the cow, in the sense that there is larger recovery of feed energy as milk energy. Sometime ago while observing the operation of milking in a large goat dairy the writer made a rough estimate that a milker, by hustling, was able to milk at the rate of about 30 pounds per hour. That is, the cost of milking was high due to the small size and output of the individual machine. On the other hand, the advantage of increasing size in this particular may largely disappear when size reaches the neighborhood of, say, 1,000 pounds live weight.

For summary of remaining points all breed classes may be combined.

Energy yield increases with size of cow in a linear manner and at the rate of about 250 pounds of 4 per cent milk per year for each 100 pounds increase in weight. Since this is about half the corresponding figure found in advanced registry records and the average yield also about half, it appears that the weight-yield relation is similar in the two classes of records if level of yield is used as a base.

The theoretical coefficient of efficiency decreases with increasing weight. Interpreted in terms of feed cost this should mean an increase in feed cost per unit of milk with size of cow, amounting to about $0.03 per hundred-weight of 4 per cent milk for each 100 pounds increase in live weight.

The recorded feed cost per hundred-weight of 4 per cent milk decreases with live weight of cow in the range 600 to 800 pounds; and is practically constant in the range 800 to 1,600 pounds.

The cause of the discrepancy between the theoretical efficiency and recorded feed cost cannot be determined from the data at hand. It is suggested that it may be due to systematic errors in the bookkeeping which tend to overcharge the small cow and undercharge the large cow for feed energy actually consumed, particularly in the matter of pasture.

REFERENCES

(1) Jordan, Whitman Howard 1917 The Feeding of Animals. (Revised edition.) The Macmillan Company.

(2) Gaines, W. L. 1928 An efficiency formula for dairy cows. Science, lxvii, 353–354.

(3) Gaines, W. L. 1928 The energy basis of measuring milk yield in dairy cows. Ill. Agr. Exp. Sta. Bul. 308.

(4) McDowell, J. C. 1930 Within the breed the big dairy cows excel. U. S. D. A. Circ. 114.

(5) Brody, Samuel, Ragsdale, Arthur C., and Turner, Charles W. 1923 The rate of growth of the dairy cow. III. The relation between growth in weight and increase of milk secretion with age. Jour. Gen. Phys., vi, 21–30.

(6) Turner, Charles, W. 1929 The relation between weight and fat production of Guernsey cattle. Jour. Dairy Sci., xii, 60–73.

(7) Ross, H. A., Hall, H. F., and Rhode, C. S. 1923. The feed cost of milk and fat production as related to yields. Ill. Agr. Exp. Sta. Bul. 244.

POSTSCRIPT

Since this paper was turned in for publication an opportunity has occurred in connection with another study, to test in a rough way the postulated relative undercharge to the large cow during the pasture season. The data which have become available are from certain herd books of members of Dairy Herd Improvement Associations in Illinois, during 1927–1929. These books show for each cow a monthly account of milk and fat yield, and cost of feed. The month of calving is given, and there is also a figure for the live weight of the cow for part of the records.

As bearing on the present discussion, selection was made of all those cows with live weight given which calved in December (84 cows), January (116 cows) and February (91 cows). Weight classes were set up by 100 pound intervals (range 600 to 1500 pounds) and the average feed cost per hundredweight of 4 per cent milk determined for the months of April and June. The regressions of feed cost on weight are somewhat irregular but may be fairly represented by straight lines. The method of least squares, weighted by the frequencies, gives the following equations for feed cost in cents per hundred-weight of 4 per cent milk: where W is live weight of cow in hundred-weights.

$$\begin{aligned}\text{April} &= 109.6 - 0.942W\\ \text{June} &= 90.9 - 2.218W\end{aligned}$$

The greater slope of the June curve may be attributed to the use of pasture, and the charge made for it, in that month. There can be little doubt that the system of bookkeeping gives an advantage to the large cow during the pasture season, which results in an apparent lower feed cost of production. The real feed cost, as between cows of different weights, can not be determined from the reported figures of the pasture season. Neither is it at all certain that the winter feed cost figures are entirely free of a defect similar to that prevailing in summer as between cows of different weights. The writer is inclined to the view that the discrepancy in results noted above (page 21) is due to faults in the feed cost figures, rather than to faults in the figures of the nutritive requirements for milk production.

BROMCRESOL GREEN PAPER IN THE MANUFACTURE OF GRAIN-CURD CASEIN*

E. O. WHITTIER

Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture

In recent work on grain-curd casein, it was found that the end-point of the precipitation,—pH 4.1—may be simply, quickly, and accurately determined by the use of bromcresol green paper. The paper was prepared by dipping S. & S. 589, White Ribbon, filter paper into a 0.4 per cent solution of bromcresol green in 25 per cent ethanol, the solution being adjusted to pH 4.6, potentiometrically measured. The papers were than dried and cut into strips of the usual test paper size. In making a test, a strip of the indicator paper is wet with the whey and stuck on a window pane away from the direct rays of the sun. The color of the paper, viewed by transmitted light, should be compared at once with permanent dry paper standards viewed by reflected light. The protein error is very slight—not more than 0.1 pH unit in whey—and turbidity may be disregarded.

Standards were obtained by comparison of color tests on buffer solutions with Ridgeway's (1) and Maerz and Paul's (2) color standards. Several observers checked each standard. The identifying numbers for each standard selected are given below, together with the corresponding pH value.

	pH 4.0	pH 4.2	pH 4.4	pH 4.6	pH 4.8	pH 5.0
Ridgeway No....	23′b	25′b	27′b	29′b	31′b	35′b
Maerz and Paul No.......	19-K-1	19-I-1½	19-K-3	18-I-5	19-I-7	19-A-8

An accuracy of 0.1 pH unit has been consistently attained in testing grain-curd casein whey by this quick colorimetric method.

REFERENCES

(1) Ridgeway, R.: Color Standards and Color Nomenclature, The Author, 1912.

(2) Maerz, A., and Paul, M. R.: A Dictionary of Color, McGraw-Hill Book Co., 1930.

---

* Received for publication October 6, 1930.

OBSERVATIONS ON THE HEAT RESISTANCE OF SOME ROPY MILK ORGANISMS*

B. W. HAMMER AND R. V. HUSSONG

Iowa State College, Ames, Iowa

While outbreaks of ropiness in market milk have most often occurred with raw supplies, pasteurized milk has occasionally been involved. Outbreaks in the pasteurized product are especially serious because usually a plant selling such milk has a greater number of customers than a plant handling raw milk and also because the appearance of a conspicuous defect in a pasteurized supply tends to discredit the whole pasteurization idea.

The development of ropiness in pasteurized milk has commonly been explained on the basis of contamination from plant equipment following the heating. Recent investigations, however, indicate that old cultures of certain ropy milk organisms may resist pasteurization exposures and, accordingly, it appears that in future outbreaks of ropiness in pasteurized milk two possibilities must be considered, (*a*) contamination following pasteurization and (*b*) causative organisms that are sufficiently heat resistant to survive the pasteurization exposure used.

OBJECT

The work reported herein involved primarily a study of the effect of various exposures at temperatures used in market milk pasteurization on ropy milk organisms secured from pasteurized materials. The object was to determine whether, in the cases studied, the presence of the ropy milk organisms was more likely due to contamination following heating or to their heat resistance. Since the ropy milk organisms secured from the pasteurized materials were all *Bacterium viscosum*, three cultures producing ropiness in milk and belonging to the Escherichia-Aero-

---

* Received for publication June 30, 1930.

bacter group were also studied from the standpoint of their heat resistance at milk pasteurization temperatures.

HISTORICAL

Harding and Prucha (1) studied the heat resistance of ropy milk organisms from a number of outbreaks of ropy milk. The tests were carried out in Sternberg bulbs and the materials heated included bouillon suspensions of twenty-four-hour agar slope cultures, eighteen-hour milk cultures that were ropy and sterile milk to which growth from twenty-four-hour agar slope cultures had been transferred by means of water. The organisms used were largely *Bacterium viscosum* but representatives of the *Bacterium aerogenes* group were also included. These investigators concluded that, ". . . . it seems clear that proper pasteurization of milk at 140° to 145° for thirty minutes should destroy any ropy milk organisms which may be in the milk."

In a study of the comparative resistance of young and mature bacterial cells to various factors, Sherman and Albus (2) noted that the young cells were more rapidly destroyed by heating to relatively low temperatures than were the older cells.

Wolff (3) investigated the influence of the age of milk on the results of pasteurization and reported data showing the effect of heating to 62° to 63°C. for 30 minutes on the bacterial content of milk when fresh and again after a holding period; both gelatin and agar plates were used. The milk pasteurized after holding regularly contained more organisms than the milk pasteurized when fresh and in some, but not all, of the instances the percentage of the organisms which survived pasteurization was greater with the milk that had been held than with the fresh milk.

Stark and Stark (4) studied the thermal death rates of young and mature cells of *Streptococcus fecalis* and of "two species of capsulated bacteria" isolated from ropy milk and found the young cells were markedly less resistant than the mature cells. These investigators point out that, "Of practical interest is the fact that the thermal death rates of the young and old cells are so different that such commercially important organisms as the 'ropy milk' bacteria may survive pasteurization or be entirely eliminated, depending upon the physiological age of the cells."

Sherman, Stark and Stark (5) found that milk kept at temperatures (e.g., 15.5°C.) permitting bacterial growth showed a greater percentage destruction of bacteria during pasteurization than similar milk when fresh or after holding at temperatures (e.g., 4.4°C.) too low to allow multiplication of the bacteria involved. In their summary they state that, "It has been established that some important milk bacteria (e.g., 'ropy milk' types) may be entirely eliminated from milk by pasteurization when the cells are in a young and growing condition, while the old cells of the same organisms are able to withstand the process."

Scales (6) investigated an outbreak of ropy milk and found that the cells present in milk held at low temperatures were more resistant to pasteurization than cells that had been allowed to develop for a short time. He pointed out that the efficiency of the pasteurizing process was increased by holding the milk at 58°F. for 45 minutes before the milk was pasteurized.

METHODS

Six heating trials were carried out, each involving a given temperature for various periods. Except in trials 1 and 6, organisms from more than one source were employed so that generally a trial included a considerable number of individual exposures.

The cultures of ropy milk organisms used were grown in milk, in diluted milk (1 part milk to 9 parts distilled water) or on beef infusion agar slopes for varying lengths of time. The diluted milk was intended to approximate the growth conditions encountered in equipment left with considerable quantities of milk solids and water in it.

All the heat exposures were made in milk, using a milk culture direct (trial 1 only) or adding material from a culture in diluted milk or on an agar slope to the milk shortly before the heating. Usually sterilized milk was employed but trial 2 was carried out with aseptically drawn milk since it was thought the physical effect of sterilization might be a factor in the heat resistance of an organism in milk.

The cultures were exposed to heat in thin walled test tubes (about 8 mm. inside diameter and about 75 mm. long) or in capillary pipettes. When the test tubes were used, 2 cc. of the milk containing one of the organisms were sealed in each tube in trials 1 and 2, while 1 cc. was used per tube in the other trials; the sealed tubes were from 45 to 55 mm. long. With the capillary pipettes an amount of the milk estimated at from one-fortieth to one-twentieth of a cubic centimeter was drawn into a pipette and the lower end then sealed.

The heating was carried out in a large water bath supported on tripods; the temperature could be kept constant and uniform within 0.2°C. of that desired by the proper regulation of the flames beneath and occasionally stirring. When the heating involved only sealed tubes, these were tied together in bunches and each bunch carried to the desired depth in the water by means of a weight. The pipettes were held in the water by fitting them into slits sawed in a board that could be placed across the top of the water bath; the water was deep enough so that the surface was several inches above the top of the milk in the pipettes. When both a pipette and a sealed tube were to be used with a given exposure, the tube was attached to the lower end of the pipette by means of a rubber band and the pipette then held in the usual way.

In the later trials (trials 4, 5 and 6) the sealed tubes or capillary pipettes containing the milk to be heated were plunged into the water bath within a very few minutes of the completion of their preparation. The procedure in the earlier trials varied; in trials 1 and 2 the tubes used were sealed and then held in ice water until all were ready to be heated, while in trial 3 the sealed tubes were held at room temperature until they were all prepared.

As soon as a heating exposure was completed, the sealed tube or pipette used was plunged into cold water so that the temperature of the milk containing the organisms would fall rapidly.

The survival or destruction of an organism under study was determined by inoculating the heated milk into sterile litmus milk, holding at room temperature and observing for growth after various periods up to one week. The sealed tubes were opened by scratching with a file and then touching with a hot glass rod, and 0.5 cc. of the heated milk was employed. The lower end of each capillary pipette was held for a few minutes in a 5 per cent solution of $HgCl_2$ in 95 per cent alcohol and, after wiping with sterile cotton, the tip of the pipette was broken off and the milk blown out as completely as possible. In the tests with aseptic milk growth of organisms other than the ropy milk types added was occasionally noted after comparatively long holding periods. The organisms involved were present in the aseptically drawn milk and were so distinctly different from the types being studied that there was no uncertainty as to the results.

In trials 1 and 2 counts were made on some of the milk ready for heating in order to secure an idea of the number of organisms present per cubic centimeter. The results are as follows:

Trial 1, culture A, 7 hour milk culture 7,300,000 per cubic centimeter, 55 hour milk culture 1,505,000,000 per cubic centimeter, and 103 hour milk culture 1,060,000,000 per cubic centimeter; trial 2, culture A, milk plus 7 hour diluted milk culture 267,000 per cubic centimeter, milk plus 79 hour diluted milk culture 12,100,000 per cubic centimeter and milk plus 79 hour agar slope culture 61,500,000 per cubic centimeter; trial 2, culture B, milk plus 7 hour diluted milk culture 254,000 per cubic centimeter, milk plus 79 hour diluted milk culture 6,325,000 per cubic centimeter and milk plus 79 hour agar slope culture 79,000,000 per cubic centimeter.

RESULTS OBTAINED

Series A

The ropy milk organism used in series A was isolated from a sample of pasteurized milk sent to the Iowa Agricultural Experiment Station from a neighboring state by a plant having trouble with ropiness in their pasteurized product. The milk was shipped without ice and was sour on arrival. Cultures causing pronounced ropiness in milk were easily isolated by direct plating and also by inoculating some of the milk into sterile milk, holding at about 7°C. until ropiness developed and then plating. Beef infusion agar and an incubation of two days at room temperature were used. All the cultures secured were of one type which was identified as *Bacterium viscosum*.

TABLE 1

Heat resistance of Bacterium viscosum from ropy pasteurized milk

	CULTURES USED		TEMPERATURE OF HEATING	PERIODS OF HEATING	SURVIVALS	
					Sealed tubes	Capillary pipettes
			°C.	minutes		
Trial 1	A and B	7 hours in diluted milk	62.8	10, 20, 30	None	
	A and B	55 hours in diluted milk	62.8	10, 20, 30	None	
	A and B	103 hours in diluted milk	62.8	10, 20, 30	None	
Trial 2	A and B	7 hours in diluted milk	62.8	10, 20, 30	None	
	A anb B	79 hours in diluted milk	62.8	10, 20, 30	None	
	A and B	79 hours on agar	62.8	10, 20, 30	None	
Trial 3	A and B	6 hours in diluted milk	62.8	10, 20, 30	None	
	A and B	103 hours in diluted milk	62.8	10, 20, 30	None	
	A and B	103 hours on agar	62.8	10, 20, 30	None	
Trial 4	A and B	6 hours in diluted milk	62.8	5, 10	None	None
	A	30 hours in diluted milk	62.8	5, 10	None	None
	A and B	54 hours in diluted milk	62.8	5, 10	None	None
	A and B	6 hours on agar	62.8	5, 10	None	None
	A	30 hours on agar	62.8	5, 10	None	None
	A and B	54 hours on agar	62.8	5, 10	None	None
Trial 5	A and B	6 hours in diluted milk	61.1	3, 5, 10	None	None
	A and B	54 hours in diluted milk	61.1	3, 5, 10	None	None
	A and B	6 hours on agar	61.1	3, 5, 10	None	None
	A and B	54 hours on agar	61.1	3, 5, 10	None	None

The heat resistance of the organism at temperatures commonly employed for pasteurization of market milk was studied using two of the cultures isolated, cultures A and B. The results obtained are presented in table 1. The data show that the organism from cultures of various ages in diluted milk or on agar slopes failed to survive any of the exposures, which varied from 3 minutes at 61.1°C. to 30 minutes at 62.8°C. The findings suggest that the organism causing the ropiness did not resist the heating but gained entrance to the milk after pasteurization.

TABLE 2

Heat resistance of Bacterium viscosum from pasteurized cream butter

	CULTURES USED		TEMPERATURE OF HEATING	PERIODS OF HEATING	SURVIVALS	
					Sealed tubes	Capillary pipettes
			°C.	*minutes*		
Trial 4	C and D	6 hours in diluted milk	62.8	5, 10	None	None
	C	30 hours in diluted milk	62.8	5, 10	None	None
	C and D	54 hours in diluted milk	62.8	5, 10	None	None
	C and D	6 hours on agar	62.8	5, 10	None	None
	C and D	54 hours on agar	62.8	5, 10	None	None
Trial 5	C	6 hours in diluted milk	61.1	3, 5, 10	None	None
	C	54 hours in diluted milk	61.1	3, 5, 10	None	None
	C	6 hours on agar	61.1	3, 5, 10	None	None
	C	54 hours on agar	61.1	3, 5, 10	None	None

Series B

An unusual defect in a sample of butter sent to the Iowa Butter Control Laboratory furnished the ropy milk organism used in series B. On arrival the butter showed a number of rather large moisture droplets at the surface, each of which was so distinctly ropy that filaments an inch or more in length could be drawn out with a needle. Since the butter was made from pasteurized cream, it presented the same problem, from the standpoint of the source of the ropy milk organism, as does ropy pasteurized milk. Cultures which produced a pronounced ropiness in milk were secured by plating one of the moisture droplets and also the butter after the moisture had been redistributed as thoroughly as possible, using beef infusion agar and an incubation of two days at room temperature; these were all of one type which was identified as *Bacterium viscosum.*

Two of the cultures isolated, cultures C and D, were used in heat resistance tests at the pasteurizing temperatures usually employed with market milk; the data obtained are given in table 2. Although the organism was taken from cultures of various ages in diluted milk or on agar slopes, it failed to survive any of the exposures which ranged from 3 minutes at 61.1°C. to 10 minutes at 62.8°C. The results accordingly indicate that the organism did not survive the heating of the cream but gained entrance to the cream or butter following the pasteurization.

TABLE 3

Heat resistance of Bacterium viscosum from ropy whey secured from pasteurized milk

	CULTURES USED	TEMPERATURE OF HEATING	PERIODS OF HEATING	SURVIVALS	
				Sealed tubes	Capillary pipettes
		°C.	*minutes*	*minutes*	
Trial 6	E 7 hours in diluted milk	61.1	1, 3, 5, 10, 20	1	None
	E 54 hours in diluted milk	61.1	1, 3, 5, 10, 20	1	None
	E 7 hours on agar	61.1	1, 3, 5, 10, 20	1	None
	E 54 hours on agar	61.1	1, 3, 5, 10, 20	1	None

Series C

A ropy milk organism secured from ropy whey was used in series C. The whey came from a vat of pasteurized whole milk set for the manufacture of cream cheese. After an overnight holding period the small amount of whey at the surface of the material showed a number of slightly brown areas, each approximately round and 2 to 3 inches in diameter. The whey in these areas was very ropy, while the remaining whey was not. Plates poured with the ropy whey, using beef infusion agar and an incubation of two days at room temperature, developed colonies that rapidly produced ropiness in milk into which they were inoculated. The ropy milk cultures secured appeared to be identical and were found to be *Bacterium viscosum.*

One of the isolated cultures, culture E, was used in heat resistance tests at 61.1°C. The results obtained, which are presented in table 3, show that in sealed tubes the organism survived a one minute heating period but failed to survive the longer periods (3, 5, 10 or 20 minutes), while in capillary pipettes it failed to survive the one minute exposure. It thus appears that the organism did not live through the pasteurization of the whole milk used but gained entrance following the heating.

Series D

The Escherichia-Aerobacter group has been responsible for outbreaks of ropiness in both raw and pasteurized milk and since all of the ropy milk organisms secured from the materials with which pasteurization had been used were *Bacterium viscosum,* 3 cultures producing ropiness in milk and belonging to the Escherichia-Aerobacter group were studied as to their heat resistance at common milk pasteurization temperatures. All 3 cultures were *Aerobacter aerogenes.* They had been in the stock cultures for several years and were secured in the picking of colonies from plates for the purpose of studying the general types of organisms present; the principal reason for keeping them was their ability to produce ropiness in milk. Culture F came from sour cream, culture G from a cow's mouth and culture H from sour cream.

The results obtained on the three *Aerobacter aerogenes* organisms, using various exposures at temperatures commonly employed for market milk pasteurization, are given in table 4. With culture F organisms from a young diluted milk culture failed to survive 62.8°C. for 10 minutes, while those from an old culture in diluted milk or on an agar slope survived this temperature for 10 minutes but not for 20 minutes. Culture G failed to survive any of the exposures used; in trial 3 organisms from a young culture in diluted milk or from old cultures in diluted milk or on an agar slope were destroyed at 62.8°C. for 10 minutes, which was the shortest period used, and in trial 5 organisms from young

TABLE 4

Heat resistance of Aerobacter aerogenes from various sources

	TYPE OF CULTURES USED	TEMPERATURE OF HEATING	PERIODS OF HEATING	SURVIVALS	
				Sealed tubes	Capillary pipettes
Culture F					
		°C.	*minutes*	*minutes*	
Trial 3	6 hours in diluted milk	62.8	10, 20	None	
	103 hours in diluted milk	62.8	10, 20	10	
	103 hours on agar	62.8	10, 20	10	
Culture G					
Trial 3	6 hours in diluted milk	62.8	10, 20, 30	None	None
	103 hours in diluted milk	62.8	10, 20, 30	None	None
	103 hours on agar	62.8	10, 20, 30	None	None
Trial 5	6 hours in diluted milk	61.1	3, 5, 10	None	None
	54 hours in diluted milk	61.1	3, 5, 10	None	None
	6 hours on agar	61.1	3, 5, 10	None	None
	54 hours on agar	61.1	3, 5, 10	None	None
Culture H					
Trial 2	7 hours in diluted milk	62.8	10, 20, 30	None	
	79 hours in diluted milk	62.8	10, 20, 30	10	
	79 hours on agar	62.8	10, 20, 30	10, 20	
Trial 3	6 hours in diluted milk	62.8	10, 20, 30	None	
	103 hours in diluted milk	62.8	10, 20, 30	10	
	103 hours on agar	62.8	10, 20, 30	10	
Trial 5	6 hours in diluted milk	61.1	10, 15, 20, 30	None	None
	54 hours in diluted milk	61.1	10, 15, 20, 30	10	None
	6 hours on agar	61.1	10, 15, 20, 30	None	None
	54 hours on agar	61.1	10, 15, 20, 30	10, 15, 20	10, 15, 20

or old cultures in diluted milk or on agar slopes were destroyed at 61.1°C. in 3 minutes. Culture H was the most resistant to heat of any of the cultures studied. Organisms from old cultures in diluted milk or on agar slopes regularly resisted 61.1°C. or 62.8°C. for 10 minutes and in some instances for 20 minutes, while organisms from the young cultures failed to survive these temperatures for 10 minutes. In trials 2 and 5 organisms from the old cultures on agar slopes were more resistant than those from the old cultures in diluted milk but this variation was not evident in trial 3 with the exposures used.

DISCUSSION OF RESULTS

The ropy milk organisms secured from pasteurized milk and products made from pasteurized milk or cream showed very little resistance to heat at the temperatures commonly used for milk pasteurization, although cultures of various ages grown under different conditions were employed. This indicates that some of the outbreaks of ropiness are due to contamination following heating and thus confirms the work of Harding and Prucha (1). In connection with the work of Stark and Stark (4) and Scales (6), it leads to the conclusion that ropiness in pasteurized dairy products may be due to either contamination following the pasteurization or causative organisms sufficiently heat resistant to survive the pasteurization exposure used.

The outbreak of ropiness in pasteurized milk which provided the organisms used in Series A was handled as follows: As soon as the ropiness was noticed a sample was taken from the milk of each patron and held over night. The next morning 27 of the 180 samples showed evidence of ropiness and the milk from the farms represented by the 27 samples was rejected. At the same time the rinsing of all the plant equipment, night and morning, with a solution of a chlorine compound was begun. With this procedure the ropiness in the pasteurized milk disappeared immediately. The excluded supplies were gradually taken back following sterilization of equipment at the farms and the submitting of samples that failed to develop ropiness. The plant employee who studied the outbreak concluded that the ropy milk organisms were spread from the plant to the farms.

The three *Aerobacter aerogenes* cultures studied, which produced ropiness in milk although not coming from ropy dairy products, varied in their heat resistance and two of the cultures were definitely more resistant than the *Bacterium viscosum* cultures. The results secured with these two cultures confirmed the observations of various investigators, especially those of Sherman and Albus (2) and of Stark and Stark (4), that cells from young cultures are less resistant than those from old cultures. In two of the three trials with one *Aerobacter aerogenes* culture the cells from an old agar slope culture were more resistant than those from an old diluted milk culture; it should be noted, however, that according to the counts given under "Methods" the former were present in larger numbers per cubic centimeter of the material heated than the latter.

SUMMARY

Ropy milk organisms were isolated from (*a*) an outbreak of ropiness in pasteurized milk, (*b*) butter which showed ropy moisture droplets at the surface and which was made from pasteurized cream and (*c*) ropy whey obtained from pasteurized milk set for the manufacture of soft cheese. All proved to be *Bacterium viscosum*. In heat resistance trials the organisms failed to survive 61.1°C. for 3 minutes; these trials involved primarily the exposure of organisms added to milk after growing for varying periods in diluted milk or on agar slopes, although in trial 1 young and old milk cultures were used direct.

Three cultures of *Aerobacter aerogenes* that produced ropiness in milk were studied for their heat resistance and found to vary greatly in this character. With culture F. organisms from an old diluted milk or agar slope culture survived 62.8°C. for 10 minutes but those from a young milk culture did not; with culture G organisms from either a young or old diluted milk or agar slope culture failed to survive 61.1°C. for 3 minutes; with culture H organisms from an old diluted milk or agar slope culture regularly resisted 62.8°C. for 10 minutes and in some instances for 20 minutes although organisms from young cultures failed to resist these exposures. In two trials with culture H organisms from

old cultures on agar slopes were more resistant than those in diluted milk.

The results indicate that in some instances ropiness in pasteurized milk or products made from it is due to contamination following the heating and that this possibility should be considered along with heat resistant causative organisms.

REFERENCES

(1) Harding, H. A., and Prucha, M. J. 1920. Is ropy milk becoming a more serious dairy trouble? Jour. Dairy Sci., iii, 502-521.

(2) Sherman, James M., and Albus, William R. 1923. Physiological youth in bacteria. Jour. Bact., viii, 127-139.

(3) Wolff, A. 1927 Welchen einfluss hat das Alter der Milch auf den Pasteurisationserfolg? Milchwirt. Zentbl., lvi, 233-238.

(4) Stark, C. N., and Stark, Pauline. 1929. The relative thermal death rates of young and mature bacterial cells. Jour. Bact., xviii, 333-337.

(5) Sherman, J. M , Stark, C. N., and Stark, Pauline. 1929. An unappreciated but important factor in the pasteurization of milk. Jour. Dairy Sci., xii, 385-393.

(6) Scales, F. M. 1929. Combating "rope" in dairies. Food Industries, i, 622-625.

VARIATIONS IN THE COAGULATION AND PROTEOLYSIS OF MILK BY STREPTOCOCCUS LACTIS*

L. A. HARRIMAN AND B. W. HAMMER

Iowa State College, Ames, Iowa

The variations occurring among the organisms that are included in a species and also among the cultures secured by plating what is believed to be a pure culture greatly complicate the classificaton of the bacteria. Variety and type designations have been introduced with some species to point out differences that appear to be important without over-emphasizing them, but the significance to be attached to variations is often difficult to determine.

The streptococci found in dairy products present a classification problem that is of special importance because of their extensive development in these materials, the variety of changes which they produce and the lack of well defined boundaries between the types. Even with the streptococci that produce considerable quantities of lactic acid in milk, variations in the type and rate of the changes produced, as well as in the morphology, make the development of a logical scheme of grouping very difficult.

When a supposedly pure culture of *Streptococcus lactis* is plated and colonies picked into litmus milk the cultures secured sometimes show a wide variation in their rates of coagulation. A similar variation may occur among the cultures other than the citrate fermenters that are secured when a butter culture developed by combining a *S. lactis* culture with a citrate fermenter is plated and colonies picked into litmus milk. This variation among organisms which presumably came from the same original culture but which differ so widely in the rates at which they produce changes in milk brings up the question of the relationship of these types.

OBJECT

The work herein reported was carried out to determine to what extent *S. lactis* cultures varying widely in their coagulation rates

---

* Received for publication June 30, 1930.

could be obtained from plates poured with purified strains of *S. lactis*. The rates at which milk is coagulated may be a deceptive basis on which to compare cultures because a slight difference in the amount of acid formed can result in one culture coagulating milk and another not, so the study of an additional change which, in general, varies in accordance with variations in the rate of coagulation seemed advisable. The correlation between rapid coagulation and pronounced proteolysis of milk that occurs with *S. lactis* cultures appeared to meet the requirements and, accordingly, the proteolyzing power of many of the cultures secured was investigated.

HISTORICAL

The results of various investigations have shown that at least certain strains of *S. lactis* are capable of bringing about protein decomposition in milk; these have recently been reviewed by Hammer and Patil (1).

Anderegg and Hammer (2) isolated a number of *S. lactis* cultures from various dairy products and studied their proteolytic activity in milk, with and without $CaCO_3$, using as a rule fourteen days incubation at room temperature. In general the cultures coagulating milk rapidly caused a definite proteolysis while the slowly coagulating cultures brought about little, if any, protein decomposition. Hammer and Patil (1) reported that a considerable number of *S. lactis* cultures investigated fell into two types, proteolytic and non-proteolytic. The first rapidly coagulated milk at room temperature, while the second showed considerable variation in the coagulation rate but never coagulated as rapidly at this temperature as the proteolytic type. The general correlation between the proteolytic activity and coagulation rate of *S. lactis* did not hold at 30° or 37°C. since at these temperatures some of the non-proteolytic strains coagulated milk about as rapidly as the proteolytic ones.

GENERAL PROCEDURE

The procedure used with each *S. lactis* culture consisted of plating on whey agar, picking contiguous colonies (about twenty-five) into litmus milk after there was a good development at room temperature and comparing at 21.1°C. the coagulation rates of the cultures secured directly from the plate. A pronounced difference in the rates of coagulation was checked by making transfers with a loop to tubes of litmus milk and noting the periods required for coagulation at 21.1°C. In most of the instances in which a definite difference in the coagulation rates was established, representatives of both the slowly and rapidly coagulating cultures were studied for their ability to proteolyze milk.

The original cultures were secured from various sources and were purified by plating on whey agar and picking colonies into litmus milk three times before beginning their study. Single cell isolations were not used because the primary object of the work was to determine the relationship of organisms isolated from dairy products by the plate method.

Since previous investigations at the Iowa Agricultural Experiment Station had shown that an increase in soluble nitrogen is regularly accompanied by an increase in amino nitrogen, the proteolyzing powers of a culture were studied by determining the change produced in the amino nitrogen content of milk in seven days at room temperature; $CaCO_3$ was added to the milk (before sterilization) and frequently distributed (by shaking) during the growth period since the neutralization of some of the acid in this way permits a greater growth of the organism and more extensive proteolysis by a proteolyzing type. The amino nitrogen was determined by the Van Slyke method using 10 cc. of a filtrate from the milk for a determination and limiting the foaming with 1 cc. of butyl alcohol. The amino nitrogen values reported represent the difference in milligrams between the amino nitrogen content of 10 cc. of filtrate from milk in which an organism had grown and that of 10 cc. of filtrate from an uninoculated portion of the same milk. The filtrate from the milk was obtained by the method already described by Anderegg and Hammer (2) and also by Hammer and Patil (1).

RESULTS OBTAINED

The work was divided into two parts. In Part I the original cultures were carried through litmus milk transfers and whenever plates were to be poured an unselected transfer was used. In Part II the original cultures were run through a series of platings, a rapidly coagulating culture secured from one plating being used to pour the next set of plates. The principal difference between the procedures in Part I and Part II is that in Part I after the original purification of the cultures there was no additional purification, while in Part II there was a continual purification throughout the series of platings, each culture plated having been through one more plating than the culture preceding it.

TABLE 1

Designations, sources and types of the cultures used in Part I

CULTURE	SOURCE	TYPE
A1 and A2	Sour cream	Typical *S. lactis*
B1 and B2	Sour cream	*S. lactis var. maltigenes*
C1 and C2	Stock culture	Typical *S. lactis*
F1 and F2	Fresh cow feces	Typical *S. lactis*
H1 and H2	Commercial butter culture	Typical *S. lactis*
K1 and K2	Milk	*S. lactis var. maltigenes*
K3	Stock culture	*S. lactis var. maltigenes*
M1 and M2	Milk	*S. lactis var. hollandicus*
O1 and O2	Milk	Typical *S. lactis*

Part I

In Part I 17 cultures were used; the designations, sources and types of these are given in table 1. The data show that the organisms studied came from various sources and included typical *S. lactis*, *S. lactis var. maltigenes* and *S. lactis var. hollandicus.*

With each organism 6 sets of whey agar plates were poured, using the original culture or a transfer (two to four days old) coming from the series of transfers made from it. After incubating at 21.1°C. until there was a good development, a plate showing a satisfactory distribution of colonies was selected and about twenty-five contiguous colonies picked into litmus milk.

When one or more cultures that appeared to be slower in their rates of coagulation than the original culture were secured, this slow coagulation was checked by a series of loop transfers to litmus milk using rapidly coagulating cultures from the same plate for comparison. Some of the cultures that coagulated slowly when first picked from the plates proved to be as rapid as any of the cultures, the slow original coagulation undoubtedly being due to the inoculation of a very small number of cells or some such factor, but most of the cultures coagulating slowly when first picked continued to show a delayed coagulation in subsequent inoculations.

TABLE 2

Numbers of slowly coagulating cultures obtained when about twenty-five colonies were picked from a plate—Part I

CULTURE	PLATING					
	1	2	3	4	5	6
A1	0	0	0	1	1	0
A2	0	0	0	0	0	0
B1	0	1	2	0	2	1
B2	0	1	2	0	2	1
C1	0	0	0	2	1	0
C2	0	0	0	0	1	0
F1	0	0	1	0	0	0
F2	0	0	0	0	2	0
H1	0	0	0	0	0	0
H2	0	0	0	0	0	0
K1	0	0	0	0	1	0
K2	0	1	1	1	1	0
K3	0	0	0	0	0	0
M1	0	2	2	0	2	1
M2	0	0	0	0	0	1
O1	1	1	0	2	3	1
O2	2	1	0	2	2	1

The numbers of slowly coagulating cultures that were secured from the various platings are summarized in table 2. The results show that slowly coagulating cultures were never secured in all six of the platings of an organism; they were secured in five of the platings with two organisms (12 per cent), in four of the platings with four organisms (24 per cent), in two of the platings with two organisms (12 per cent), in one of the platings with five organisms (29 per cent) and in none of the platings with four organisms. The numbers of slowly coagulating cultures secured per 25 colonies picked from a plate varied from none to three (from 0 to 12 per cent); the total numbers secured from the six platings of one organism ranged from none to eight (from 0 to 5 per cent); of the approximately 2550 cultures picked from the plates, fifty (1.96 per cent) were slow coagulators.

From each plating which yielded cultures differing definitely in their rates of coagulation, a slowly coagulating culture and a rapidly coagulating one were selected and studied for their proteolyzing power. The results obtained are given in table 3. Of the 35 pairs of cultures studied, in all but four the rapidly coagulating culture definitely gave more proteolysis than the slowly coagulating one. With these four the rapidly coagulating cultures failed to produce as extensive a proteolysis as that produced by the rapidly coagulating cultures in the other 33 comparisons.

TABLE 3

Proteolysis by rapidly and by slowly coagulating cultures—Part I

CULTURE	PLATING	INCREASE (OVER CHECK) AMINO N PER 10 CC FILTRATE		CULTURE	PLATING	INCREASE (OVER CHECK) AMINO N PER 10 CC FILTRATE	
		Rapid	Slow			Rapid	Slow
		mgm	*mgm*			*mgm*	*mgm*
A1	4	0.132	0.055	M1	2	2.007	0.145
A1	5	0.269	0.140	M1	3	1.512	0.191
B1	2	0.746	0.053	M1	5	1.456	−0.052
B1	3	0.631	0.301	M1	6	2.257	0.121
B1	5	0.798	−0.025	M2	6	0.767	0.121
B1	6	0.767	−0.007	O1	1	0.936	0.063
B2	2	0.807	0.059	O1	2	1.227	0.006
B2	3	0.989	0.163	O1	4	0.817	0.188
B2	5	−0.661	0.052	O1	5	1.088	0.026
B2	6	1.080	0.067	O1	6	0.824	0.027
C1	4	0.837	0.077	O2	1	0.626	0.081
C1	5	0.853	0.086	O2	2	0.760	−0.081
C2	5	0.054	0.027	O2	4	1.003	0.106
F1	3	1.508	0.043	O2	5	0.557	0.053
F2	5	0.038	0.091	O2	6	0.974	0.000
K1	5	1.357	0.074				
K2	2	0.882	0.110				
K2	3	1.045	0.270				
K2	4	1.061	0.021				
K2	5	1.220	0.074				

A number of pairs of cultures (each pair representing a plate) with which there was a definite difference in their rates of coagulation at 21.1°C. were compared at 30° and 37°C. but at these temperatures there was no difference between the cultures constituting a pair. This is in agreement with the observations reported by Hammer and Patil (1).

Part II

Four cultures were used in Part II, all of which were isolated from sour cream. H1 and H2 were *S. lactis var. maltigenes*, while H3 and H4 were typical *S. lactis.*

Each of the organisms was run through a series of platings on whey agar at 21.1°C., using a rapidly coagulating culture (two to four days old) from one plating as inoculating material for the next. After a satisfactory development had taken place with the plates in each set, a plate showing a good colony distribution was selected and about twenty-five contiguous colonies picked into litmus milk and incubated at 21.1°C. From some of the plates one or more cultures that were comparatively slow in coagulating milk were secured. The relative coagulation rate of each of these was checked against a rapidly coagulating culture from the same plate by making loop transfers to litmus milk. As in Part I a few of the cultures originally selected as slow proved to be rapid, while the others continued to coagulate milk slowly during a series of transfers.

Table 4 shows the numbers of cultures coagulating milk slowly that were secured from each plating of each organism. Such cultures were secured from nine (31 per cent) of the twenty-nine platings of H1, from seven (27 per cent) of the twenty-six platings of H2, from three (10 per cent) of the thirty-one platings of H3 and from eight (26 per cent) of the thirty-one platings of H4. The numbers of slowly coagulating cultures secured per twenty-five colonies picked from a plate varied from none to nine (0 to 36 per cent); of the approximately 2925 cultures picked from the plates, forty-seven (1.61 per cent) were slow coagulators.

In all but one of the platings from which slowly coagulating cultures were secured, one (occasionally more) of these cultures was compared as to proteolyzing ability with a rapidly coagulating culture from the same plate. The results are given in table 5.

TABLE 4

Numbers of slowly coagulating cultures obtained when about twenty-five colonies were picked from each plate in the regular series—Part II

PLATING	CULTURE			
	H1	H2	H3	H4
1	1	0	0	0
2	0	1	0	0
3	1	2	0	0
4	1	1	0	0
5	0	0	0	0
6	0	0	1	0
7	3	1	0	0
8	0	0	0	2
9	0	1	0	1
10	0	0	0	1
11	1	0	0	1
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
17	0	0	1	0
18	1	0	0	0
19	0	1	0	0
20	0	0	0	0
21	0	1	0	0
22	1	0	0	0
23	0	0	0	0
24	1	0	0	0
25	0	0	0	0
26	0	0	0	0
27	1		0	4
28	0		1	1
29	0		0	0
30			0	6
31			0	9

The rapidly coagulating culture in a pair always gave more proteolysis than the slowly coagulating one and usually the difference was marked.

In an attempt to increase the incidence of the slowly coagulating cultures secured from a plate, additional platings were made using a young uncurdled culture in some instances and an old culture (up to six days) in others. The general procedure followed was that already outlined. Both the young and old cultures yielded approximately the same number of slow coagulators as the cultures plated in the regular series. Some of the slowly coagulating cultures obtained from either a young or an old culture were studied for their proteolyzing power in milk and each gave much less proteolysis than a rapidly coagulating culture from the same plate.

TABLE 5

Proteolysis by rapidly and by slowly coagulating cultures—Part II

PLATING	H1		PLATING	H2		PLATING	H3		PLATING	H4	
	INCREASE (OVER CHECK) AMINO N PER 10 CC. FILTRATE			INCREASE (OVER CHECK) AMINO N PER 10 CC FILTRATE			INCREASE (OVER CHECK) AMINO N PER 10 CC. FILTRATE			INCREASE (OVER CHECK) AMINO N PER 10 CC FILTRATE	
	Rapid	Slow		Rapid	Slow		Rapid	Slow		Rapid	Slow
	mgm.	*mgm.*		*mgm.*	*mgm*		*mgm*	*mgm.*		*mgm*	*mgm*
1	1 447	0 407	2	1.043	0 165	6	1 637	0 691	8	1 632	0 182
3	1.047	0 386	3	0.967	0 030	17	0 879	0 000	9	1 548	0 116
4	1.060	−0 123	4	0 791	0 123	28	0 997	0 332	10	1 987	0 111
7	0 772	0 278	7	0 993	0 621				11	1 561	0 061
11	1 561	0 227	9	1 159	0 992				27	1.319	0 000
18	1 114	0.663	19	0 987	0 000				28	1 486	−0 056
22	1 510	0 691	21	1 129	0 797				30	1 321	0 182
27	0 932	0 274							31	1.340	0 210

A number of the slowly coagulating cultures secured were plated on whey agar and after a good colony development had occurred at 21.1°C. twenty-five contiguous colonies were picked from one plate in each set and incubated at the same temperature. The cultures obtained in this way all coagulated milk slowly. There was some variation in the coagulation rates of the colonies from a plate but this was slight and not comparable with the variation often noted among the colonies from a plate poured with a rapidly coagulating culture.

SUMMARY AND CONSIDERATION OF RESULTS

The results presented show that certain pure cultures of *S. lactis* rapidly coagulating milk can be split into rapidly and slowly coagulating strains and that, in general, the former produce considerable proteolysis in milk, while the latter bring about little or no proteolysis. The general correlation between rapid coagulation and pronounced proteolysis with *S. lactis* cultures that has been noted with isolations from various sources thus appears to persist when variations occur in the rates of coagulation of cultures picked from a plate poured with a pure culture. In Part I the percentage of the approximately 2550 colonies picked that were slow coagulators was 1.96, while in Part II the percentage of the approximately 2925 colonies was 1.61. These percentages agree quite closely with the percentage (2.4) of non-ropy cultures of *S. lactis* secured by Hammer (3) when 1288 colonies were picked from plates poured with ropy cultures.

In the trials carried out the cultures secured by plating a slowly coagulating culture and picking colonies into litmus milk were all slow in their coagulation rates. It thus appears to be much more difficult to secure rapidly coagulating cultures from slow ones than to secure slowly coagulating cultures from rapid ones.

The variations in the rates of coagulation and the extents of proteolysis among the cultures secured when plating what would ordinarily be regarded as a pure culture of *S. lactis* make these characteristics of little value in the classification of this group. They also indicate that pronounced differences in the coagulation rates of cultures secured from a plate poured with a dairy product do not *necessarily* indicate a difference in species.

REFERENCES

(1) Hammer, B. W., and Patil, V. H. 1930. Proteolysis by *Streptococcus lactis* with special reference to butter cultures and butter. Iowa Agr. Expt. Sta., Res. Bul. 123, p. 58–91.

(2) Anderegg, L. T., and Hammer, B. W. 1929. Proteolysis by *Streptococccus lactis*. Jour. Dairy Sc., xii, 114–128.

(3) Hammer, B. W. 1923. Studies on ropiness in cultures of *Streptococcus lactis*. Iowa Agr. Expt. Sta., Res. Bul. 74, p. 259–270.

VARIATIONS IN THE BUFFER VALUE OF HERD MILK*

PAUL D. WATSON

Research Laboratories, Bureau of Dairy Industry, U. S. Department of Agriculture

Interest has been directed recently to the importance of the buffer value of milk, particularly in regard to dairy manufacturing and nutritional studies. The buffer intensity of milk is a function of its acid-base equilibrium, and is especially significant in relation to the casein and phosphate systems. Whittier (1), and Holm and Webb (2) of these laboratories have already pointed out the importance of the buffer index of milk; the former has investigated its relation to various milk constituents, and the latter have indicated its significance in nutrition, especially with reference to the composition of infant foods.

Many industries are fortunate in the fact that their raw materials are very uniform in quality or may be easily controlled. However, the basic material of the dairy industry is a biological fluid, which varies markedly from time to time and from place to place, the variations being due to many factors.

Considerable information has been published concerning the variation in the constituents of milk, but little knowledge is available concerning variations in the acid-base equilibrium of milk which have an important bearing on certain processes in the manufacture of casein, cheese and condensed milk products. It was thought, therefore, that these considerations warranted the presentation of some studies which indicate the variations which may occur in the buffer capacity of milk.

THEORETICAL

The theoretical aspects of buffering, and the calculation of the buffer index, will be treated only briefly here, inasmuch as Van Slyke (3) has discussed the subject quite fully. Additional information with particular reference to milk will be found in the work of Clark (4), Buchanan and Peterson (5), and Whittier (1).

---

* Presented at the annual meeting of The American Dairy Science Association, Washington, D. C., June 26 to 28, 1929.

The buffer value of a solution may be expressed as its power to resist change in pH upon the addition or loss of acid or alkali. Experimentally, standard acid or alkali in measured amounts is added to the solution and the pH determined before and after each addition. From these data a titration curve is drawn by plotting the amount of alkali or acid used, against the change in pH produced. The buffer value at any pH may be determined graphically by drawing the tangent to the curve at that pH and determining its slope. Mathematically, if the added alkali or acid is ΔB, and the increase in pH is ΔpH, then the average buffer value over the observed pH range is $\beta = \frac{\Delta B}{\Delta pH}$.

Van Slyke (3) was the first to adopt the differential ratio $\frac{dB}{d\,pH}$ as the expression of the relation between the increment in gram equivalents per liter of acid or base added to a buffer solution and the resultant increment in pH.

The buffer index of a solution is not constant and has an exact value only at a definite pH, and therefore the value calculated over an observed pH range is an average one. In practise, a close approximation is made if the buffer index is calculated for the mean pH over a small pH interval. The area under a buffer curve between two pH values is a measure of the buffer capacity of the solution in the particular range chosen.

EXPERIMENTAL METHOD

The initial pH of the milk was determined, and then to 50 cc. portions were added 3, 5, 7, 10, and 12 cc., respectively, of 0.2 N hydrochloric acid. The acid was added from a burette to each portion in a 250 cc. Erlenmeyer flask while it was shaken vigorously. The final pH was measured after the lapse of two hours, since Whittier (1) has shown that equilibrium is attained in this time.

The hydrogen-ion concentrations were determined by means of the quinhydrone method, using gold electrodes of the capillary form (6). The potential differences against a saturated calomel half-cell were measured with a Leeds and Northrup type K potentiometer.

Titration curves were then plotted and the cubic centimeters of acid required for each change of 0.2 in pH were read therefrom. The buffer index for each 0.2 pH interval was then calculated by substitution in the formula,

$$\frac{d\,B}{d\,pH} = \frac{(\text{cc. acid added})\ (\text{normality factor})}{(\text{volume of milk})\ (\text{pH change})}$$

Van Slyke (3) states that the volume change due to the added acid or alkali may be ordinarily neglected, if the maximum increase is below 50 per cent of the original volume. Then too, the work of Buchanan and Petereson (5) indicates that milk may be diluted up to 30 per cent of its volume without appreciable effect on the buffer value. Inasmuch as the volume changes which occurred in the present work were below these limits, no correction for them was considered necessary in the above formula.

A number of determinations were made over the pH range between 4.5 and 6.5 in order to locate the region of maximum buffering; typical curves are shown in figure 1. It was found that the maximum buffer values occurred between pH 5 and 6, and consequently most of the determinations were limited to this zone.

DESCRIPTION OF MILK

The milk on which these buffer studies were made came from the herd at the Bureau of Dairy Industry Experiment Station at Beltsville, Maryland. The herd consisted of approximately 25 Jersey and 25 Holstein cows, but the milk received was not always from the same cows each day. The samples taken for the determinations on the Jersey and Holstein milk represented about 550 pounds of milk, and the samples taken of the mixtures of these milks represented about 1100 pounds.

The samples were obtained after the milk had been clarified, standardized, and thoroughly mixed for the purpose of Swiss cheese manufacture. The standardization was done by separating some of the fat from a portion so that the ratio of fat to casein was about 1:7. Inasmuch as fat is a negligible factor in buffering, change in the percentage of it would not have an appreciable effect upon the buffer values.

The initial pH of the milk samples ranged from 6.50 to 6.64.

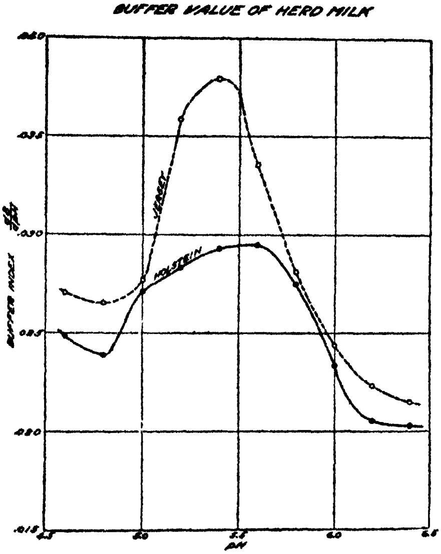


FIG. 1

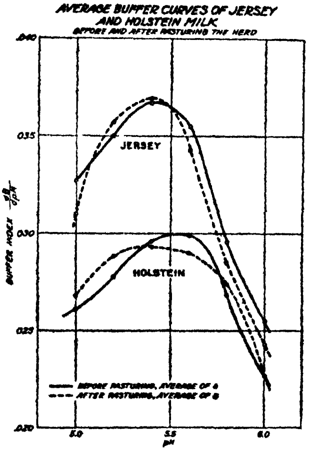


FIG. 2

DIFFERENCE IN THE BUFFER VALUE OF JERSEY AND HOLSTEIN MILK AND THE EFFECT OF PASTURING THE HERD

The experiments of some investigators (7) (8), indicate that the change in rations occurring when cows are pastured, results in an increase in those milk constituents of importance in buffering, such as protein and ash, while from a review of other researches (9), it may be concluded that changes in ration are without significant effect upon these milk constituents. Some determinations were made of the buffer value of Holstein and Jersey milk before and after the herd was pastured. These results are presented in tables 1 and 2, and the average values are plotted

TABLE 1

Buffer values of Jersey and Holstein milk before the herd was pastured

pH ZONE	FEBRUARY 11, 1929		FEBRUARY 12, 1929		FEBRUARY 13, 1929		FEBRUARY 14, 1929		FEBRUARY 15, 1929		FEBRUARY 16, 1929		FEBRUARY 18, 1929		APRIL 22, 1929		APRIL 23, 1929	
	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein
4.7–4.9		0.0293		0.0257		0.0222		0.0220		0.0234		0.0228		0.0224	0.0227	0.0277	0.0401	0.0251
4.9–5.1	0.0339	0.0251	0.0297	0.0277	0.0369	0.0265	0.0335	0.0241	0.0353	0.0259	0.0351	0.0265	0.0301	0.0259	0.0311	0.0261	0.0289	0.0273
5.1–5.3	0.0371	0.0257	0.0351	0.0281	0.0343	0.0289	0.0341	0.0271	0.0351	0.0275	0.0351	0.0305	0.0343	0.0269	0.0351	0.0283	0.0347	0.0275
5.3–5.5	0.0373	0.0319	0.0363	0.0285	0.0351	0.0295	0.0359	0.0313	0.0359	0.0281	0.0355	0.0293	0.0387	0.0285	0.0375	0.0293	0.0381	0.0297
5.5–5.7	0.0361	0.0313	0.0365	0.0283	0.0347	0.0293	0.0359	0.0327	0.0357	0.0283	0.0367	0.0287	0.0337	0.0303	0.0335	0.0299	0.0369	0.0301
5.7–5.9	0.0307	0.0255	0.0299	0.0255	0.0309	0.0281	0.0293	0.0249	0.0303	0.0267	0.0291	0.0293	0.0299	0.0271	0.0301	0.0277	0.0265	0.0265
5.9–6.1	0.0251	0.0227	0.0257	0.0237	0.0259	0.0233	0.0253	0.0218	0.0253	0.0228	0.0253	0.0214	0.0271	0.0220	0.0245	0.0227	0.0255	0.0243

TABLE 2

Buffer values of Jersey and Holstein milk after the herd was pastured (April 23, 1929)

pH ZONE	APRIL 24, 1929		APRIL 25, 1929		APRIL 26, 1929		APRIL 27, 1929		APRIL 29, 1929		APRIL 30, 1929		MAY 2, 1929		MAY 3, 1929	
	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein
4.7–4.9		0.0261		0.0244		0.0251	0.0265	0.0239		0.0259	0.0261	0.0241		0.0222		0.0224
4.9–5.1	0.0333	0.0281	0.0321	0.0271	0.0363	0.0271	0.0277	0.0271	0.0317	0.0277	0.0299	0.0255	0.0279	0.0261	0.0289	0.0257
5.1–5.3	0.0389	0.0309	0.0361	0.0285	0.0367	0.0299	0.0359	0.0283	0.0371	0.0305	0.0329	0.0291	0.0359	0.0277	0.0317	0.0257
5.3–5.5	0.0411	0.0315	0.0375	0.0289	0.0363	0.0315	0.0379	0.0293	0.0377	0.0299	0.0343	0.0305	0.0377	0.0259	0.0329	0.0269
5.5–5.7	0.0325	0.0307	0.0361	0.0287	0.0351	0.0321	0.0335	0.0295	0.0321	0.0289	0.0367	0.0297	0.0335	0.0251	0.0347	0.0275
5.7–5.9	0.0319	0.0267	0.0277	0.0283	0.0287	0.0269	0.0281	0.0275	0.0279	0.0253	0.0307	0.0303	0.0275	0.0291	0.0255	0.0251
5.9–6.1	0.0244	0.0220	0.0238	0.0220	0.0253	0.0234	0.0245	0.0233	0.0255	0.0228	0.0243	0.0233	0.0238	0.0246	0.0228	0.0200

in figure 2. It is evident that after the herd was pastured there was a small shift of the peak of the buffer values to the left (possibly indicating a small change in the milk constituents), but the effect was slight, the marked deviation in buffer index being due to the difference in breed.

In connection with the difference in the buffer values of Holstein and Jersey milk, it is interesting to note that there also exists a difference in the hardness of the curd of milk from the two breeds, which is of practical significance in nutrition and the dairy industries. Hill (10), who studied the physical curd character of milk in its relation to digestibility for infants, found that Holstein milk was much softer in curd character than Jersey milk, on the average. These results have been corroborated in these laboratories by Mr. K. J. Matheson. Then too, Holm and Webb (2) have pointed out that cow's milk in order to simulate the action of mother's milk in addition to yielding a fine soft curd,

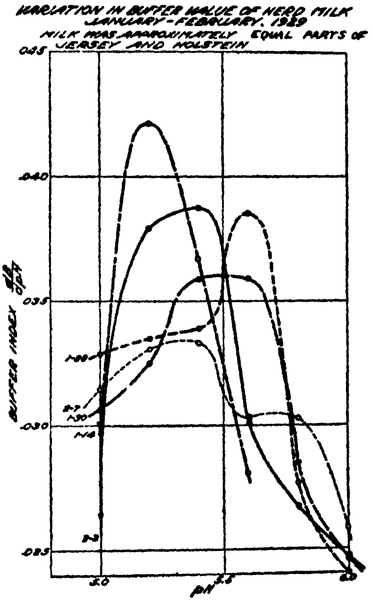


FIG. 3

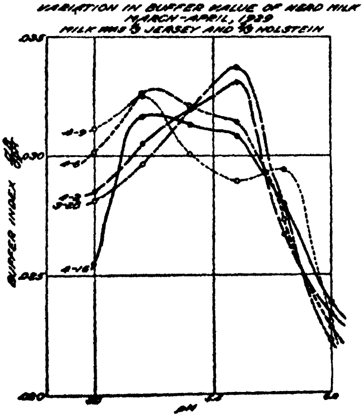


FIG. 4

TABLE 3

Buffer value of herd milk

Milks were a mixture of approximately one-half Jersey and one-half Holstein

pH ZONE	JANUARY 28, 1929	JANUARY 29, 1929	JANUARY 30, 1929	JANUARY 31, 1929	FEBRUARY 1, 1929	FEBRUARY 2, 1929	FEBRUARY 4, 1929	FEBRUARY 6, 1929	FEBRUARY 7, 1929	FEBRUARY 8, 1929	FEBRUARY 9, 1929	AVERAGE
4.9–5.1	0.0329	0.0293	0.0307	0.0319	0.0321	0.0265	0.0295	0.0313	0.0315	0.0269	0.0309	0.0303
5.1–5.3	0.0335	0.0315	0.0325	0.0377	0.0333	0.0379	0.0315	0.0357	0.0331	0.0387	0.0357	0.0346
5.3–5.5	0.0339	0.0343	0.0359	0.0357	0.0337	0.0387	0.0361	0.0351	0.0333	0.0379	0.0347	0.0354
5.5–5.7	0.0385	0.0359	0.0359	0.0321	0.0335	0.0301	0.0421	0.0333	0.0303	0.0333	0.0363	0.0347
5.7–5.9	0.0277	0.0319	0.0285	0.0279	0.0297	0.0273	0.0305	0.0313	0.0303	0.0295	0.0275	0.0293
5.9–6.1	0.0241	0.0261	0.0247	0.0255	0.0255	0.0247	0.0253	0.0253	0.0257	0.0249	0.0249	0.0252

TABLE 4

Buffer value of herd milk

Milks were a mixture of one-third Jersey and two-thirds Holstein in exact proportion

pH ZONE	MARCH 28, 1929	MARCH 30, 1929	APRIL 1, 1929	APRIL 2, 1929	APRIL 5, 1929	APRIL 6, 1929	APRIL 9, 1929	APRIL 12, 1929	APRIL 13, 1929	APRIL 15, 1929	APRIL 16, 1929	APRIL 19, 1929	AVERAGE
4.9–5.1	0.0281	0.0273	0.0289	0.0285	0.0277	0.0301	0.0311	0.0289	0.0273	0.0287	0.0255	0.0265	0.0282
5.1–5.3	0.0297	0.0327	0.0357	0.0305	0.0299	0.0327	0.0325	0.0309	0.0333	0.0309	0.0317	0.0321	0.0320
5.3–5.5	0.0319	0.0325	0.0327	0.0319	0.0305	0.0321	0.0301	0.0311	0.0313	0.0303	0.0313	0.0329	0.0316
5.5–5.7	0.0337	0.0309	0.0287	0.0331	0.0291	0.0315	0.0289	0.0319	0.0285	0.0311	0.0309	0.0333	0.0308
5.7–5.9	0.0277	0.0259	0.0263	0.0267	0.0267	0.0273	0.0295	0.0287	0.0287	0.0289	0.0279	0.0257	0.0275
5.9–6.1	0.0222	0.0228	0.0234	0.0234	0.0234	0.0228	0.0230	0.0232	0.0228	0.0230	0.0238	0.0243	0.0232

should have a low buffer capacity, the first point being the more important. It is apparent that if Jersey milk were diluted so the solids-not-fat content would be the same as in Holstein milk, then the buffer capacity of the two milks should not be markedly different. Furthermore, in the field of nutrition, the curdling properties of milk may be more important than the buffer value (2). Then too, the difference between the hardness of the curd of Jersey milk and Holstein milk would probably be less if the protein concentration of the milks were equalized.

The average initial pH of the Jersey milk was 6.56 as compared with 6.58 pH for the Holstein milk. The fact that the difference between the pH of the milks was small indicates that there is no correlation between the buffer value and the hydrogen-ion concentration of milk.

VARIATION IN THE BUFFER VALUE OF MIXTURES OF JERSEY AND HOLSTEIN MILK

In table 3 are some buffer values of milk received in January and February. This milk was mixed at the farm, and was approximately half Jersey and half Holstein milk. Some of the results are shown graphically in figure 3; the curves serve to indicate the variation in buffer indices which are likely to occur from day to day in a milk supply.

A number of experiments were made in which the buffer capacity was determined on milks which were composed of exactly one-third Jersey and two-thirds Holstein milk, the proportions being accurately determined in the laboratory. These results are shown in table 4, and some are plotted in figure 4. It will be noted that daily variations in the buffer value also occurred when the proportions of the two milks were determined exactly. The decreased percentage of Jersey milk accounted for the lower buffer value of these milks, the average of the peaks falling at about 0.032, whereas in the previous experiment the average value was above 0.035.

SUMMARY

Jersey milk was found to have a markedly greater buffer capacity than Holstein milk, in the pH zone of 5 to 6.

Pasturing the herd had a negligible influence upon the buffer value of the milk.

The extent of the variations in buffer value which are likely to occur in a milk supply are indicated.

REFERENCES

(1) Whittier, E. O. 1929 Buffer intensities of milk and milk constituents. Jour. Biol. Chem., lxxxiii, 79.

(2) Holm, G. E., and Webb, B. H. 1930. The buffer capacities of various milks and proprietary infant foods. Am. Jour. Diseases Children, xl, 260.

(3) Van Slyke, D. D. 1922 On the measurement of buffer values and on the relationship of buffer value to the dissociation constant of the buffer and the concentration and reaction of the buffer. Jour. Biol. Chem., lii, 525.

(4) Clark, W. M. 1927 "Synthetic milk" as a basis for research. Jour. Dairy Sci., x, 195.

(5) Buchanan, J. H., and Peterson, E. E. 1927 Buffers of milk and buffer value. Jour. Dairy Sci., x, 224.

(6) Watson, P. D., 1927 Use of quinhydrone electrode for following changes of pH in Swiss cheese. Ind. and Eng. Chem., xix, 1272.

(7) Pelekhov, N. 1926 Changes produced in milk under the influence of change from dry feeding to pasture and from additions of calcium salts. Zapiski Belorusskoi Gosudarstv. Akad. Sel'skogo Khozyaistva l, 1.

(8) Davies, R. O., and Provan, A. L. 1928 The relation of food to the composition of milk. Welsh Jour. Agr., iv, 114.

(9) Maynard, L. A. 1929 The influence of the nature of the ration of the cow upon the nutritive value of milk. Cornell Veterinarian, xix, 124.

(10) Hill, R. L. 1928 The physical curd character of milk and its relationship to the digestibility and food value of milk for infants. Utah Agr. Expt. Sta. Bull. 207.

VIABILITY OF LACTOBACILLUS ACIDOPHILUS AND LACTOBACILLUS BULGARICUS CULTURES STORED AT VARIOUS TEMPERATURES*

LUTHER A. BLACK

Division of Dairy Husbandry, Washington Agricultural Experiment Station, Pullman

Investigators have found that a large number of living acidophilus organisms must be ingested in order to secure beneficial results from the consumption of acidophilus preparations. According to Kopeloff (1), about 1000 cc. of milk containing 200 million viable acidophilus organisms per cubic centimeter should be ingested daily. The therapeutic effect was found to decrease when the number of living organisms was less than 100 million per cubic centimeter and only 1000 cc. of the milk was ingested each day. It is desirable therefore, that acidophilus preparations should originally contain a large number of living bacteria, and that the product should be used before there is a decided decrease in numbers.

The majority of commercial products that have been offered to the public have been unsatisfactory. Analysis (2) (3) of such commercial products has suggested that the preparations either do not originally contain a sufficiently large number of the organisms or else too long a time has elapsed between production and consumption. Since a comparatively small amount of the commercial culture is consumed daily, the presence of large numbers of acidophilus organisms is essential.

Milk cultures of *Lactobacillus acidophilus* are desirable, in that the organisms grow readily in milk and the resulting product is usually more palatable and nutritious than other forms of acidophilus products. The necessary numbers of acidophilus organisms are present in a properly prepared fresh milk culture.

---

* Received for publication July 17, 1930. Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 166, College of Agriculture and Experiment Station, State College of Washington.

In many places the consumption of acidophilus milk is not sufficient to warrant its preparation each day. Consequently, these cultures may be prepared once or twice a week. This necessitates storage of the acidophilus culture for varying periods of time before its distribution. In addition the cultures are kept in the home for a time before the product is entirely consumed. For these reasons the acidophilus preparations may be several days old before they are consumed.

Personal experience, as well as the experience of others, indicates that cultured milks are more palatable if they are consumed when they are cold. When a milk culture is kept at room temperatures, it is less palatable. In some cases this is due to the slight caramelization caused by sterilization of the milk before inoculation. This caramelized flavor is less noticeable in a chilled product. In many instances a slight caramelization of the milk is not at all apparent in cold acidophilus milk. A "chalky" flavor has also been noticed in warm cultured milks just as they were taken from the incubator. This flavor disappeared upon cooling of the milk below room temperature.

When cultured milks of the proper acidity are kept for a time at refrigerator temperatures, they retain their desirable, mildly acid flavor. At room temperatures, the acidity of the culture increases and so causes the milk to be more acid to the taste than is desirable. In addition, there is more "wheying off" of the milk, resulting in a product of less desirable appearance. If the culture is kept for several days, the acidity may increase sufficiently to make the milk unfit for consumption. When the acidity becomes too high a diminution of the desired organisms also results. Since a large number of organisms is necesary, this action is detrimental.

Results showing a great loss in numbers when acidophilus milk was kept in the refrigerator have frequently been quoted in the recent literature on *L. acidophilus*. For this reason, it has been recommended that acidophilus products be stored at room temperatures. If the organisms die rapidly at refrigerator temperatures, keeping the product at cold temperatures would retain palatability but would result in a loss of therapeutic properties. In keeping the product at warmer temperatures, a less palatable product would result, with the probability of lessened consumption.

Results that show a marked reduction in the number of viable organisms when acidophilus milk is kept at refrigerator temperatures, thus assume considerable importance. Such results are also contrary to the accepted effects of low temperatures upon bacterial life.

HISTORICAL

Kopeloff and Cheney (4) inoculated 3 flasks of sterilized milk with *L. acidophilus* and after incubation at 37°C. for 24 hours, the flasks were held at 25° to 30°C. for two weeks. They reported that the number of viable organisms diminished rapidly after 3 days, accompanied by a corresponding rise in acidity. The milk became less palatable and had a very unpleasant taste by the end of two weeks. Similar results were obtained upon repetition of the experiment.

In further experiments Kopeloff (1) inoculated 3 flasks, each containing 300 cc. of sterilized skim milk, with 10 cc. of culture per flask. The flasks were incubated at 35°C. for 24 hours, and then placed in an ice box. The number of viable *L. acidophilus* was determined each day by plating a portion of the milk. After one day in the ice box, 46 per cent of the viable organisms had died; after 2 days, 73; and after 3 days, 89 per cent of the original number were not viable. Kopeloff concluded that it is more desirable to keep *L. acidophilus* products at room temperature than at ice box temperatures.

Reichart and Davis (5) also presented some counts on acidophilus milk stored at various temperatures. When held at 2° to 15°C. the number of organisms in acidophilus milk was found to decrease considerably. In later experiments there was little decrease in number of organisms during the first 24 hours storage at 24°C. and in two cases there was an increase. During the second 24 hours there was approximately a 50 per cent decrease at 24°C. At 4°C. there was a decided decrease, as was the case at 37°C. It was concluded that 21°C. was better for storing acidophilus milk than refrigerator temperatures. The storage of acidophilus milk for longer than 48 hours, even at room temperature, was found to result in a product containing few *L. acidophilus* organisms.

PROCEDURE

The cultures of *L. acidophilus* and *L. bulgaricus* used in the following experiments were secured from our stock cultures or from the collections of other investigators. Culture A1, A4, and B6 were stock cultures. Culture A15 was a recently isolated fecal strain. These cultures were transferred into tubes of sterile milk at 24-hour intervals for a few days preceding their use.

Separated milk of a low bacterial content was sterilized at 15 pounds pressure for 25 minutes. After cooling, a flask was inoculated with 1 per cent of a 24-hour milk culture of *L. acidophilus.* The flask was then placed at 37°C. for incubation until there was evidence of coagulation. The increase in acidity was determined at intervals by withdrawing a sample for titration. The flasks were removed from the incubator after coagulation took place or when the acidity reached a predetermined amount.

A sample was withdrawn for plating from the well mixed culture. A portion of the culture was then placed into each of 4 sterilized flasks, stoppered with cotton. One of these flasks was placed at each of the different temperatures used, 37°, 22°, 9° and 0°C. These cultures were held at the respective temperatures for one week. Samples for plating and for acidity were withdrawn every 24 hours.

The medium used for all plating was Bacto dehydrated whey agar. Duplicate plates were poured and incubated at 37°C. for 5 days. The dilutions were made in rubber-stoppered, glass bottles containing an amount of distilled water determined by experiment to be 99 cc. after sterilization. The use of a solid stopper permitted thorough shaking of the samples, a procedure essential for breaking up the coagulated milk. In the majority of the experiments, only one dilution (1:1,000,000) was made.

A 10 cc. sample was used for the acidity titration. Straight walled glass tubing, marked to contain 10 cc., or inverted 10 cc.

pipettes, similarly marked, were satisfactory for measuring the coagulated milk for the acidity test.

Samples for the acidity test were withdrawn from the well stirred sample by submerging only slightly the bottom of the sampling pipette, and drawing the milk just up to the mark. In this way, very little of the culture remained on the outside of the pipette or inside the pipette above the mark. It was necessary to rinse out all of the milk within the pipette. The simplest way to do this seemed to be to use the pipette as a stirring rod during the titration of its respective sample. After diluting the milk by the addition of alkali, the pipette could be well rinsed with the alkali and milk mixture. A more rapid titration was also possible, inasmuch as alkali could be rapidly added to slightly past the endpoint. Then, by rinsing out the remaining culture in the pipette, the reaction would again be acid and the endpoint could then be carefully approached.

PRESENTATION OF DATA

In a preliminary experiment on *L. acidophilus*, culture A15, a flask of the culture was placed in storage at 0°C. After two days the count had decreased from 1075 million to 820 million organisms per cubic centimeter.

This result was interesting, inasmuch as two days storage at 0°C. had reduced the living organisms less than 25 per cent.

The results of 8 experiments at intervals over a period of 7 days are given in tables 1 and 2. The cultures in these tables are grouped by culture number, and not in order of the experiment.

Minor fluctuations in the percentage of acidity sometimes appeared. These were slight and probably were largely due to the approximate measurements secured with the sampling tubes marked to contain 10 cc.

Considerable fluctuations were secured in some of the *L. acidophilus* counts. While the fact that the bacteria may be clumped may account for some fluctuation, yet other factors are concerned. The fluctuations seemed to be due to failure of the organisms to grow as colonies on the media and under the conditions provided. That they were present in the culture and viable,

TABLE 1

The acidity and count of L. acidophilus milk cultures stored at the temperatures indicated

CULTURE	STORAGE	0°C.		9°C.		22°C.		37°C.	
		Acidity	Count	Acidity	Count	Acidity	Count	Acidity	Count
	days	*per cent*	*millions*	*per cent*	*millions*	*per cent*	*millions*	*per cent*	*millions*
A1	0	0.50	88.0*	0.50	88.0*	0.56	88.0*	0.56	88.0*
	1	0.58	21.0	0.59	31.0	0.72	84.0	1.08	46.5
	2	0.58	30.5	0.61	109.0	0.73	†	1.40	0.5
	3	0.59	131.0	0.60	106.0	0.72	100.0	1.40	0.0
	4	0.58	28.0	0.61	97.0*	0.75	146.5	1.41	0.0
	5	0.59	74.5	0.58	73.5	0.86	113.0	1.47	0.0
	6	0.66	107.0	0.63	99.0*	0.86	124.0*	1.48	0.0
	7	0.59	8.0*	0.63	54.0	0.93	38.5	1.53	0.0
A1	0	0.80	118.0	0.80	118.0	0.80	118.0	0.80	118.0
	1	0.81	166.5	0.83	137.0	1.04	125.5	1.41	75.0
	2	0.81	93.5	0.85	119.5	1.18	127.5	1.69	67.0
	3	0.81	73.5	0.83	†	1.22	108.5	1.81	44.0
	4	0.81	59.5	0.85	118.0	1.24	83.0	1.86	38.0
	5	0.81	53.5	0.85	135.5	1.27	108.0*	1.96	13.5
	6	0.81	68.5	0.85	106.0*	1.29	94.0	1.96	8.0
	7	0.81	56.5	0.87	64.5	1.37	66.0*	2.07	5.0
A1	0	0.78	50.5	0.78	50.5	0.78	50.5	0.78	50.5
	1	0.75	65.0	0.78	53.0*	1.01	59.5	1.40	55.5
	2	0.78	42.0	0.84	41.5	1.06	42.5	1.62	34.0
	3	0.78	39.0*	0.85	38.0	1.16	†	1.79	15.0
	4	0.77	36.0*	0.85	37.5	1.21	51.5	1.90	4.0
	5	0.78	56.5	0.85	43.5	1.24	38.5	2.02	1.5
	6	0.78	39.0*	0.86	47.5	1.29	43.0	2.08	1.0
	7	0.78	44.0	0.86	31.0	1.35	16.5	2.16	0.0
A4	0	0.79	135.5	0.79	135.5	0.79	135.5	0.79	135.5
	1	0.81	16.5	0.81	130.0	1.00	285.0	1.31	188.0
	2	0.81	138.0	0.83	255.0*	1.01	45.0	1.55	0.0
	3	0.81	329.0	0.82	324.0	1.03	239.0*	1.61	0.0
	4	0.80	257.0	0.82	291.0*	1.04	200.5	1.66	0.5
	5	0.78	20.0*	0.77	148.5	1.10	185.0*	1.80	0.0
	6	0.80	107.0	0.86	46.5	1.19	217.5	1.71	0.0
	7	0.79	44.5	0.86	113.0	1.19	3.5	1.72	0.0
A15	0	0.80	1,035.0	0.80	1,035.0	0.80	1,035.0	0.80	1,035.0
	1	0.80	705.0	0.85	1,105.0	0.95	1,835.0	1.37	2,140.0
	2	0.83	985.0	0.85	1,205.0	1.03	2,160.0	1.59	1,605.0
	3	0.80	960.0	0.81	1,335.0	1.06	1,775.0	1.96	1,630.0
	4	0.79	745.0	0.94	1,300.0	1.31	1,900.0	2.11	1,820.0
	5	0.78	985.0	0.94	1,380.0*	1.40	2,115.0	2.37	1,055.0
	6	0.78	990.0*	0.95	1,020.0	1.42	1,415.0	2.31	505.0
	7	0.79	680.0	1.01	1,195.0	1.51	2,000.0*	2.32	56.5

* Result of one plate.

† No count, usually spreaders.

is demonstrated by their appearance in increased numbers on days following those in which the count was low. The fact that higher counts are secured after low counts is a positive indication of the presence of the organisms.

TABLE 2

The acidity and count of L. bulgaricus milk cultures stored at the temperatures indicated

CULTURE	STORAGE	0°C.		9°C.		22°C.		37°C.	
		Acidity	Count	Acidity	Count	Acidity	Count	Acidity	Count
	days	*per cent*	*millions*	*per cent*	*millions*	*per cent*	*millions*	*per cent*	*millions*
B6	0	0.83	221.5	0.83	221.5	0.83	221.5	0.83	221.5
	1	0.85	†	0.85	251.0	0.92	162.0	1.48	265.0
	2	0.84	345.0	0.86	324.5	0.90	198.0	1.78	0.5
	3	0.85	265.5	0.86	314.5	0.92	208.5	1.85	1.5
	4	0.84	236.0*	0.86	322.5	0.92	262.0	1.91	0.0
	5	0.83	291.0	0.83	399.0	0.90	317.5	1.93	0.0
	6	0.83	226.0*	0.83	311.5	0.95	398.0*	2.00	0.0
	7	0.83	188.5	0.86	187.0	0.93	176.0	2.00	0.0
B6	0	0.71	155.5	0.71	115.5	0.71	155.5	0.71	115.5
	1	0.71	46.0	0.73	111.0	0.86	108.0	0.98	10.0
	2	0.70	41.5	0.74	74.0	1.01	81.0	1.04	1.0
	3	0.70	22.5	0.73	58.5	0.99	46.5	1.11	1.5
	4	0.71	18.0*	0.75	71.5	0.99	37.5	1.08	3.0
	5	0.71	9.5	0.76	56.5	0.99	33.0	1.15	3.0*
	6	0.70	11.5	0.78	39.5	1.00	9.0*	1.15	2.5
	7	0.71	6.0	0.79	13.0	1.01	4.5	1.20	2.0
B6	0	0.63	201.5	0.63	201.5	0.63	201.5	0.63	201.5
	1	0.59	162.5	0.68	200.0	0.78	221.5	1.16	136.0
	2	0.63	167.5	0.77	207.5	0.90	180.5	1.40	77.0*
	3	0.65	†	0.74	200.5	0.93	229.0*	1.52	42.0*
	4	0.65	198.0	0.73	222.0	0.99	220.0	1.58	92.5
	5	0.65	206.0	0.76	229.5	1.08	233.5	1.65	96.0
	6	0.65	193.5	0.78	216.5	1.15	208.5	1.75	71.5
	7	0.65	123.0	0.82	109.0	1.16	149.0	1.86	27.5

* Result of one plate.

† No count, spreaders or molds.

Apart from the fluctuations in plate counts of the acidophilus organisms, very definite trends are indicated with respect to the change in numbers of viable organisms at the different temperatures. This may perhaps be more apparent from the summary presented in table 3. This table indicates the viable organisms present, expressed in percentage of the original number before storage.

According to the results of the experiments, as shown in tables 1, 2, and 3, there is only a slight reduction in the numbers of viable organisms upon holding cultured milks at refrigerator temperatures, even during a period of one week. As a matter of interest, storage of these products at as low a temperature as 0°C. did not result in the diminution of organisms to the extent reported by other investigators as a result of storage at refrigerator temperatures.

TABLE 3

Summary of the effect of storage of L. acidophilus milk upon the number of organisms

Results expressed as percentage of original count before storage

DAYS OF STORAGE	0°C				9°C				22°C				37°C.			
	Average			Average all strains	Average			Average all strains	Average			Average all strains	Average			Average all strains
	A1	A4	A15		A1	A4	A15		A1	A4	A15		A1	A4	A15	
1	97	12	68	74	85	95	106	91	106	210	117	141	75	138	206	114
2	65	101	95	78	102	118	116	108	96	33	208	106	41	0	155	55
3	96	242	92	124	97	239	128	132	102	176	171	131	22	0	157	44
4	51	189	71	83	94	214	125	124	112	147	183	134	13	0	175	43
5	80	14	95	80	94	109	133	105	98	136	204	127	4	0	101	23
6	85	78	95	86	98	34	98	85	101	160	136	120	2	0	48	11
7	48	32	65	48	59	83	115	75	44	2	193	65	1	0	5	1

There was very little difference in the percentage decrease, whether stored at room or refrigerator temperatures. However, the product held in the refrigerator retained its palatability and keeping quality, increasing only slightly in acidity. The product kept at room temperature increased considerably in acidity, with a consequent loss in palatability and keeping quality. Furthermore, the room temperature used was constantly 22°C., a lower temperature than would be maintained in the average room, particularly in summer. At temperatures of 37°C., a high acidity was soon reached and the number of viable organisms was quickly reduced, resulting in a loss of therapeutic properties, keeping quality and palatability.

Comparison of L. acidophilus and L. bulgaricus

In table 4 the average of three experiments on *L. acidophilus* is compared to the average of three experiments on *L. bulgaricus*. The results given in table 4 indicate that *L. acidophilus* and *L. bulgaricus* are similar in their ability to remain viable in milk cultures when held at the temperatures indicated.

TABLE 4

A comparison of the effect of storage upon L. acidophilus and upon L. bulgaricus

Results expressed as percentages of original count before storage

DAYS OF STORAGE	AVERAGE 3 TRIALS L. ACIDOPHILUS, STRAIN 1				AVERAGE 3 TRIALS L. BULGARICUS, STRAIN 6			
	0°C	9°C	22°C	37°C	0°C	9°C	22°C	37°C
1	97	85	106	75	55	94	84	64
2	65	102	96	41	88	99	67	13
3	96	97	102	22	44	93	79	7
4	51	94	112	13	72	100	83	15
5	80	94	98	4	79	110	93	16
6	85	98	101	2	68	91	96	12
7	48	59	44	1	49	62	52	5

The storage of an L. acidophilus commercial broth culture

In the results already presented, only milk cultures have been concerned. One analysis made on a commercial broth preparation of *L. acidophilus* is also of interest in connection with the temperature of storage. According to the label, the organism used was predominately an X strain, recently isolated from the human intestinal tract. This culture was regularly purchased from a laboratory specializing in these cultures.

According to the label, the preparation examined was manufactured November 1, 1929. The number of viable organisms at the time of manufacture was stated to be 200,000,000 per cubic centimeter. The expiration date was December 1, 1929. The estimated number of viable organisms at the date of expiration was stated to be 100,000,000 per cubic centimeter. The label also stated that the preparation should be kept at room temperature.

This product was plated out on November 4 and again on November 14. At this latter time the preparation was divided, a portion being placed in the refrigerator 9°C. (48°F.) and the remainder left at room temperature, 20° to 24°C. (68 to 75°F.). The results obtained are presented in table 5.

The results obtained with the broth culture indicate again that refrigerator temperature is equal or superior to room temperature for the storage of *L. acidophilus*.

TABLE 5

Counts obtained upon a commercial broth culture of L. acidophilus

DATE EXAMINED	TEMPERATURE OF STORAGE	PLATE COUNT
November 4, 1929	Room	48,000,000
November 14, 1929	Room	31,850,000
December 1, 1929	Room	3,605,000
	Refrigerator since November 14, 1929	15,150,000

Storage temperature for laboratory cultures

Still further evidence might be brought forward regarding the effect of refrigerator temperature upon *L. acidophilus*. Our stock cultures are kept at this temperature and transferred not more than once a month, unless they are needed for some special purpose.

In addition to stock cultures which might have adapted themselves to the conditions under which they are kept, similar results were obtained with a recently isolated fecal strain. Several sealed tubes of this X type recently isolated fecal strain, single cell cultures, were received in November 1928. One tube was opened at that time and used in early experiments. The other tubes were placed in the refrigerator. In November, 1929, a second sealed tube of this set which had been in the refrigerator approximately a year, was opened. The contents, 2 or 3 cc., placed in 10 cc. of sterile milk coagulated the milk within 24 hours.

DISCUSSION

The rapid decrease in the number of living organisms in milk cultures of *L. acidophilus* that has been found by other investigators is believed to be largely due to the acid formed. In the report of Kopeloff and Cheney (4) with cultures held at room temperature, the acidity was given. The acidity reported for the first, second, and third days was 1.00, 0.85, and 0.95 per cents respectively, with counts of 177, 184, and 141 millions. These investigators stated that the acidity rapidly increased thereafter, accompanied by a rapid decrease in the number of living organisms. In the work reported by Kopeloff (1) the acidity of the samples when placed in the refrigerator was not given. However, a $3\frac{1}{3}$ per cent inoculation with a 24 hour incubation period was used. In the present investigation a 1 per cent inoculation produced about 0.8 per cent acid in 30 hours. The acid produced within a given time will depend upon the strain, its activity, amount of inoculation, time and temperature of incubation, and temperature of the milk when inoculated.

Reichart and Davis (5) did not report the acidity of the samples that they used. In the directions these investigators gave for the preparation of acidophilus milk, an inoculation with 1 to 4 per cent was used, followed by incubation for 18 to 20 hours or until a slight amount of whey was formed. The acidity at that time was stated to be from 1 to 1.5 per cent. These investigators found that acidophilus milk held at 40°F. showed a decided decrease in living organisms, during the first 24 hours, as did that held at 98°F. Acidophilus milk held at 75°F. showed only a slight decrease in 24 hours. In two cases there was an increase in numbers. Storage longer than 48 hours, even at room temperature, was found to cause a decided decrease in numbers.

According to the results of the present investigation and other unreported results, the earlier observations just mentioned can at least be partially explained upon the basis of acidity. After a certain acidity is reached, the organisms seem to die rapidly. If a sample of cultured milk reached the acidity at which a noticeable decrease in numbers began and was then divided and placed at various temperatures, the following might be expected.

At 37°C. there would be a rapid increase in acidity and a high death rate, offsetting any earlier increase in numbers. At 22°C., a slower increase in acidity would be expected, with continued death of organisms, but with a multiplication of organisms sufficient to slightly increase or nearly maintain the original number or to decidedly decrease the reduction in numbers. At 9°C. some decrease in numbers, due to the acidity already reached, would continue for a while. This decrease might largely take place during the time required to thoroughly chill the culture, and the decrease would probably be greater due to additional acid produced before the formation of acid entirely ceased. While some multiplication of organisms might occur during the cooling process, there would not be the multiplication present that occurs at 22°C. or above. The decrease in numbers due to acidity, associated with the lack of multiplication at 9°C., might result in apparently a more rapid decrease at 9°C. than at 22°C.

Observations indicate that the acidity preferred by the consumer of cultured milks seems to be not more than 0.80 to 1.00 per cent. When the milk is consumed, which may be several days after its manufacture, there will have been some increase in acidity. This increase in acid depends upon the time the product was exposed to higher temperatures during delivery, the temperatures at which it was stored where manufactured and the time and temperature at which it was held in the home before consumption. In order to have a product not too high in acidity when consumed, incubation of the milk should cease at the lowest acidity that results in a desirable acid flavor and at the same time yields a sufficient number of living acidophilus organisms.

The demand for acidophilus milk sometimes is not sufficient to warrant its preparation each day. Even if the product is made each day, some time elapses before it is consumed. In the home, the milk may not be used immediately but may be consumed over a period of a day or two. In order to secure keeping quality for such a length of time, the product must be held at refrigerator temperatures. A recommendation of room temperature for storage would result in keeping the product at a variable temperature between 22° and 37°C. depending upon seasonal or other conditions. Such a temperature would impair the keeping qualities and palatability, and in addition would cause a more rapid reduction in organisms than would occur at refrigerator temperatures.

The results reported demonstrate that storage at refrigerator temperatures is the most satisfactory from the standpoint of numbers, as well as acidity. Other unpublished results and observations have also indicated that this is true.

Some of the counts on acidophilus milk reported in the various tables are lower than is desirable for therapeutic purposes. All plates in the experiments reported upon were incubated under atmospheric conditions. This might result in a lower plate count than the number of viable organisms actually present, since investigators (6) (5) (3) have shown that incubation in a carbon dioxide atmosphere results in higher plate counts. It was also intended that the cultures should be placed in storage at an acidity low enough so the acid formed would not have any destructive effect on the chilled cultures. It is probable that the cultures were removed from the incubator before the maximum number of organisms had been reached.

The different strains seem to vary somewhat in numbers. One strain gave an unusually high count. This may have been due to the fact that it was more recently isolated. On the other hand, this characteristic has been maintained for over a year as a stock culture. The same strain may vary from time to time in rapidity of coagulation and in numbers of organisms. Repeated transfers usually increase the rapidity of coagulation and increase the numbers of organisms present.

SUMMARY

1. Milk cultures of *L. acidophilus* have been stored at refrigerator temperatures for several days without causing any marked reduction in the number of living organisms.

2. Less reduction in numbers of *L. acidophilus* occurred at refrigerator temperature, 9°C. (48°F.), or even at 0°C. (32°F.), than occurred at 37°C. (99°F.).

3. There was little difference in viability between organisms in

acidophilus milk kept several days at refrigerator temperature, 9°C. (48°F.), and at 22°C. (72°F).

4. Judging from the results obtained at 9° and 0°C. it seems probable that cultured milk could be held at the usual storage temperatures of 4.4°C. (40°F.) or below for several days without greatly affecting the number of viable acidophilus organisms.

5. The various strains of *L. acidophilus* examined reacted in a similar manner to the different storage temperatures.

6. The strain of *L. bulgaricus* examined reacted to the storage temperatures in a manner similar to *L. acidophilus.*

7. The stock cultures of *L. acidophilus* and the recently isolated fecal strain used in the experiments were alike in their reactions to the storage temperatures.

8. Results of one acidophilus commercial broth culture indicated that refrigerator storage was also preferable to storage at room temperatures for that product.

9. Stock cultures of *L. acidophilus* whether old or recently isolated strains, remained viable when held in the refrigerator and transferred once in 4 to 6 weeks or longer. In one case a sealed tube of a recently isolated fecal strain stored at refrigerator temperatures for one year was viable when removed.

REFERENCES

(1) Kopeloff, N. 1923 Some temperatures studies on *L. acidophilus* milk. State Hospital Quarterly (N. Y.), viii, 372–381. As reprinted in *Lactobacillus acidophilus*, p. 197–203, 1926. The Williams and Wilkins Company.

(2) James, L. H. 1922 *Bacillus acidophilus* and *Bacillus bulgaricus* cultures and preparations: bacteriologic status. Jour. Amer. Med. Assoc., lxxix, 89–92.

(3) Kopeloff, N., Cohen, P., and Beerman, P. 1928 Commercial acidophilus products. Jour. Amer. Med. Assoc., xci, 1187–1192.

(4) Kopeloff, N., and Cheney, C. O. 1922 Studies on the therapeutic effect of *Bacillus acidophilus* milk and lactose; preliminary communication. Jour. Amer. Med. Assoc., lxxix, 609–612.

(5) Reichart, E. L., and Davis, H. P. 1928 The commercial application of *Lactobacillus acidophilus* milk. Nebraska Agr. Exp. Sta. Bul. No. 228.

(6) Kulp, W. L. 1926 The determination of viable *Lactobacillus acidophilus.* Science, lxiv, 304–306.

CORRELATION BETWEEN ANNUAL BUTTER FAT PRODUCTION AND ANNUAL FEED COST OF DAIRY COWS UNDER FARM CONDITIONS*

C. W. McINTYRE

Dairy Extension Specialist, Jackson County, Independence, Missouri

The fact that cows having a large annual milk and butterfat production are more profitable than low producers is widely recognized by dairymen and dairy workers.

Eckles and Reed (1) found that two Jersey cows raised under identical conditions of feed and care varied greatly in their ability to economically produce milk and butterfat. The better cow produced 3.9 pounds of butterfat for each pound produced by the other cow, during their first two lactations. During their third lactation the better cow produced 2.77 pounds of fat for each pound produced by the inferior cow. During this year the better cow consumed 1.75 pounds of feed for each pound consumed by the poorer cow. They found that when dry, the better cow required more feed for maintenance. The percentage of feed digested by the better cow was slightly higher than that digested by the poorer cow, but the difference was not enough to explain the difference in production.

Cooper, Bennett and Church (2) found that as the annual production of dairy cows increased there was a relatively uniform increase in the annual feed cost. As the annual production per cow increased, there was a corresponding decrease in the feed cost of a unit of production. They found this decrease greater between poor and fair cows than between fair and good cows, also a greater decrease between fair and good cows than between good and excellent cows.

Bronson (3) observed that the annual feed cost of dairy cows increased as the annual milk and fat production increased.

North (4) reported that the annual feed cost per cow was greater on farms having a higher annual production per cow.

---

* **Received for publication July 17, 1930.**

He also found that the feed cost per unit of production was lower on farms having a higher annual production of milk and fat per cow.

According to Erf (5) the feed cost per unit of product decreased when the annual production per cow increased. Data from the Cornell Station (6) shows that the cost of feed per unit of product is greater when the annual production is low for each cow.

Ross, Hall and Rhode (7) at Illinois found a correlation of −0.4570 ±0.0133 between the annual butterfat production of a cow and the total digestible nutrients consumed for each pound of butterfat produced. The correlation was −0.4180 ±0.0139 between the annual milk production per cow and the digestible nutrients consumed for each 100 pounds of milk. They found that the amount of concentrates used per pound of fat was constant but the amount of succulence and dry feed consumed for each unit of product was higher as the annual production decreased. The quantity of digestible nutrients required for each pound of fat decreased as the annual production per cow increased.

Henry and Morrison (8) point out that the production of a cow is dependent upon the feed she receives. A good cow on full feed uses only one half her ration for body maintenance and the remaining half for milk production. A cow receiving a half ration, has food only for body maintenance, and will produce little or no milk. A cow on three-quarters ration has enough feed to produce about one-half her normal flow of milk. A cow that is "over fed" produces her normal flow of milk and uses the extra feed for gain in weight.

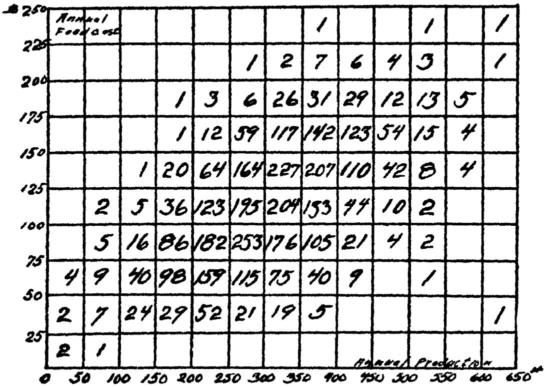
Converse (9) was able to increase the milk and fat production of a dairy cow by feeding an excess of digestible nutrients. However the increase in production was not in exact proportion to the increase in digestible nutrients.

Dairy Herd Improvement records published in Missouri and other states all show that the higher producing cows are the more profitable ones, but little is said concerning the higher cost of feeding them.

The object of this work is to determine the correlation, if any, between the annual butterfat production of a cow and the cost of feed for one year, regardless of the kind of feed and care the animal receives. Butterfat, instead of Milk Production is used because as Marshall (10) states "The yield of butterfat tends to constancy, the yield of milk having a negative correlation to the fat test."

METHOD OF PROCEDURE

All data used were taken from the records of the Jackson County, Missouri, Dairy Herd Improvement Association, filed in the County Agrents office. These records from 79 herds, include 3844 individual yearly cow records covering a period of six years, 1923 to 1929. They were from herds of as few as 8 cows to those of over 200 and include cows of all ages. The average production of the herds ranged from 160 to 395 pounds of butterfat per cow for one year. Some were purebreds, while others were grades. Holsteins and Jerseys predominated with Guernseys and Shorthorns present in smaller numbers. Some herds were well fed and cared for while others were poorly managed. Some were farm herds, others were commercial dairy herds,



							1			1		1
					1	2	7	6	4	3		1
			1	3	6	26	31	29	12	13	5	
			1	12	59	117	142	123	54	15	4	
		1	20	64	164	227	207	110	42	8	4	
	2	5	36	123	195	204	153	44	10	2		
	5	16	86	182	253	176	105	21	4	2		
4	9	40	98	159	115	75	40	9		1		
2	7	24	29	52	21	19	5					1
2	1											

CHART 1. ORIGINAL DATA CLASSIFIED ACCORDING TO PRODUCTION AND FEED COST

Average production of 3844 cows was 307.7 ±0.96, pounds standard deviation, 87.95 ±0.67 and the coefficient of variation, 28.58 ±0.237. The average feed cost was $112.16 ±0.42, the standard deviation 38.25 ±0.30 and the coefficient of variation 34.10 ±0.291.

fed purchased hay and grain with no home grown feed or pasture. The herds were located in every section of the county and are a representative sample of the herds in the county.

The records of all cows, in a herd 10 months or more of the testing year, were included in these data, regardless of the length of time the animal was dry.

They were then divided into groups as shown in chart 1. The records were classified for production and feed cost, 50 pounds of butterfat being the class interval for production and $25.00 the class interval for the annual feed cost. Frequency distribution and regression graphs were made. Correlation and other figures were all calculated by the methods given by Yule (11).

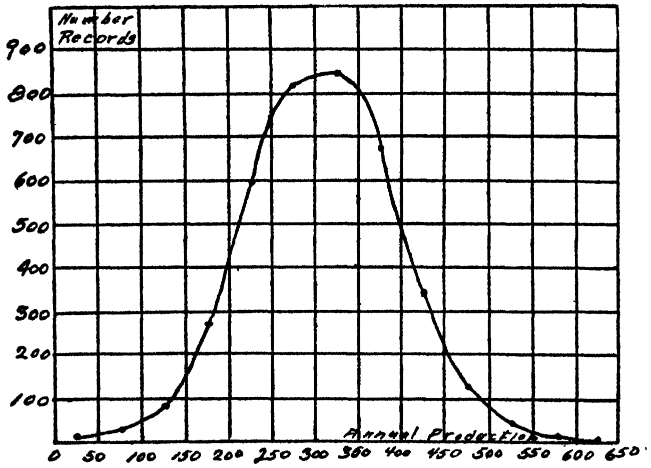


Fig. 1. Frequency Distribution of Individual Records According to the Annual Butterfat Production of the Individual Cows

PRESENTATION OF DATA

The original data are all presented in chart 1 showing the number of records in each of the class intervals both by production and feed cost. The frequency distribution by production is practically normal (fig. 1). The frequency distribution by feed cost (fig. 2) is more nearly a combination of two normal curves, rather than one curve. This probably is due to combining the records, from commercial dairies that buy all their feed and have a high average feed cost with records from farm herds that buy no hay and little grain and have a low average feed cost.

The average feed cost for each of the groups according to production (table 1) was found to range from $43.75 a year for the group that produced less than 50 pounds of fat annually, to $164.43 for the group that produced above 550 pounds and less than 600 pounds of fat. The feed cost was lower for the group that produced over 600 pounds of fat, probably due to the small number of records in this class. The average production according to feed cost (table 2) ranged from 41.6 pounds of fat annually in the group that cost less than $25.00 to feed one year, to 508.3 pounds of fat annually in the group that cost over $225.00 to feed a year. The range or variation in each of these groups was very uniform as shown by the regression graph (fig. 3) showing the average

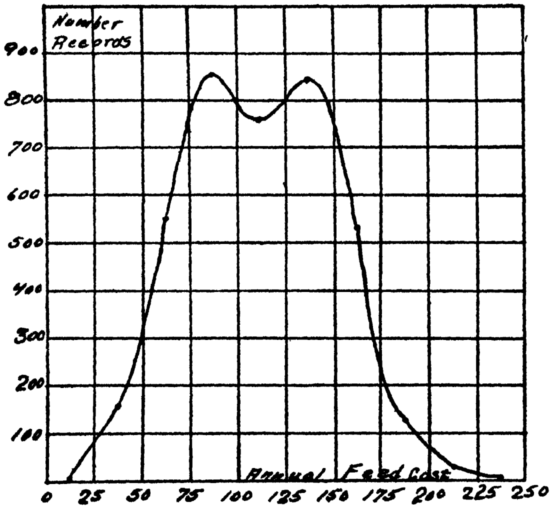


FIG. 2. FREQUENCY DISTRIBUTION OF INDIVIDUAL RECORDS ACCORDING TO THE ANNUAL FEED COST OF THE INDIVIDUAL COWS

feed cost by production and the average production by feed cost. None of the averages vary greatly from the normal line of regression.

TABLE 1

Average feed cost by production

PRODUCTION	NUMBER	ANNUAL FEED COST	FEED COST PER 1 POUND FAT
pounds			
0–50	8	$43.75	$1.75
50–100	24	63.25	0 833
100–150	86	63 07	0.505
150–200	271	80 86	0 462
200–250	595	89.01	0 396
250–300	814	105 07	0.382
300–350	846	118 24	0.364
350–400	671	128.04	0 341
400–450	342	143.79	0 338
450–500	126	152.58	0.321
500–550	45	162.50	0.310
550–600	13	164.43	0.286
600–650	3	162.50	0 259
Average......307 7		$112.16	36.4¢
Total.......... . . .	3.844		

TABLE 2

Average production by feed cost

FEED COST	NUMBER	AVERAGE PRODUCTION
$0–25	3	41.6
25–50	160	217.5
50–75	550	232.8
75–100	850	278.1
100–125	754	303.8
125–150	847	339.7
150–175	527	376.7
175–200	126	403.2
200–225	24	425.0
225–250	3	508.3
Average....$112.16		307.7
Total..................	3,844	

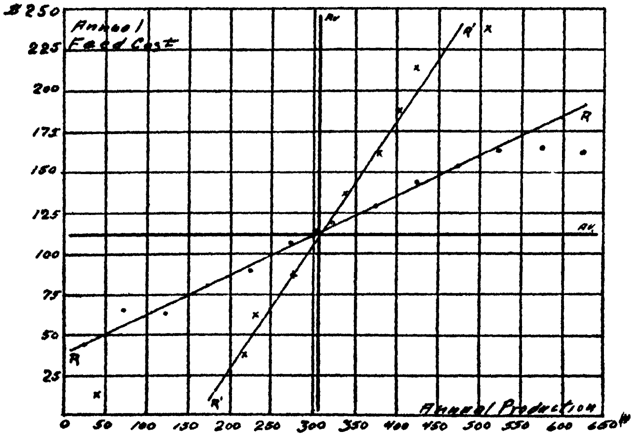


FIG. 3. CORRELATION BETWEEN YEARLY BUTTERFAT PRODUCTION AND ANNUAL FEED COST OF DAIRY COWS

Means of the rows are shown by crosses and means of the columns by dots. RR and R′R′ are lines of regression. The correlation $\gamma = +0.5641 \pm 0.007$.

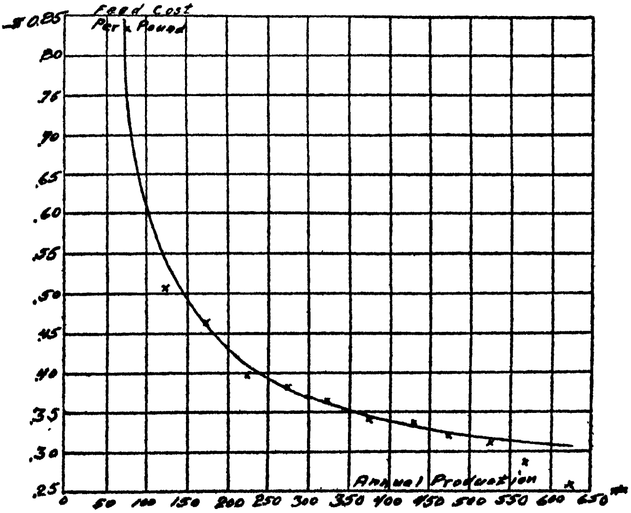


FIG. 4. VARIATION IN THE AVERAGE FEED COST OF 1 POUND OF FAT FROM COWS OF DIFFERENT PRODUCTIVITY

The data show that the feed cost of 1 pound of fat decreased as the annual production per cow increased (fig. 4 and table 1). They show definitely that the feed cost decreases rapidly as the production increases up to 400 pounds of fat annually. When yearly production is above 400 pounds of fat the decrease, in the cost of feed for each pound, is much smaller in proportion to the increase in production.

The increase in annual feed cost is smaller in proportion to the increase in production when the annual production is low. This is shown in table 3 which is a table calculated by using the regression equation. The feed cost was 40.1 per cent greater, for cows that produced 200 pounds of fat annually than it was for those that produced only 100 pounds. The feed for cows that produced 600 pounds of fat cost 66.7 per cent more than the feed for those that produced only 300 pounds of fat in a year.

TABLE 3

Increase in feed cost when production is doubled

PRODUCTION	FEED COST*	DOUBLE PRODUCTION	FEED COST*	INCREASED COST	PER CENT INCREASE
pounds		*pounds*			
100	$61 21	200	$85 74	$24 53	40 1
150	73 47	300	110 27	36 80	50 1
200	85 74	400	134 80	49.06	57 2
250	98 01	500	159 33	61 32	62 6
300	110 27	600	183 86	73.59	66 7

* These feed cost figures are calculated values obtained by the use of the regression formulae given in this paper but check very closely with the actual values given in table 1.

The average yearly fat production of the 3844 cows was 307.7 ±0.96 pounds with a standard deviation of 87.95 ±0.67 and a coefficient of variation of 28.58 ±0.237 per cent. The average annual feed cost was $112.16 ±0.42 with a standard deviation of 38.25 ±0.30 and a coefficient of variation of 34.10 ±0.291 per cent.

The correlation between the annual fat production and the yearly feed cost was 0.5641 ±0.007. The regression equations are:

$$x = 0\ 2453\ y + 36\ 68$$
$$y = 1\ 296\ x + 162\ 3$$

When x is the annual cost of feed in dollars and y is the annual production in pounds of fat. Figure 3 shows the correlation between the annual fat production of a cow and the cost of her feed for the year.

DISCUSSION OF RESULTS

These records show a definite correlation between the feed cost and the annual butterfat production of a dairy cow. They show that this correlation exists in spite of a wide variation in the productive ability of the cows, the type of feed they are fed, the care and management they receive, the age of the cows and to a certain extent the market value of the feeds. These are true because of the great variation in the herds from which the data are collected and the six-year period over which the records extend.

The feed costs found in table 3 were obtained by use of the regression formula. These values are used because they represent the normal line of regression and vary but little from the actual feed cost in the various groups. This lack of any great deviation is shown in figure 3.

Data in table 3 show there is a smaller proportionate increase in feed cost when the production of a poor herd is doubled than when the production of a fair or good herd is doubled.

Use of the regression formula $x = 0.2453\ y + 36.68$ indicates that it costs an average of $36.68 a year for feed to maintain a dairy cow and additional feed worth $24.53 for each 100 pounds of butterfat she produces during the year. The probable explanation of this is that all cows are fed what hay and other roughage they will eat and are then fed grain in direct proportion to the amount of milk or butterfat they produce. This statement will also explain the smaller decrease in the feed cost of one pound of fat, and the larger proportionate increase in annual feed cost when improvement is made in the better classes of dairy stock.

The very uniform and normal distribution of the data is indicated by the curves in figures 1 and 2 and by figure 3 which shows the close relation of the lines of regression and the distribution of the averages which determine these lines.

CONCLUSIONS

1. A positive correlation exists between the annual fat production and annual feed costs of dairy cows, under farm conditions
2. The feed cost of 1 pound of butterfat is lower when the annual production of a cow is greater.
3. This proportionate increase in feed cost is greater when the production of good cows is doubled, than when the production of poor cows is doubled.
4. These data indicate that cows require certain feed for maintenance, above which the feed cost for each hundred pounds of butterfat produced annually will be practically the same whether a cow is a light or heavy producer.
5. These data indicate that the high producing cow is more profitable because the cost of maintenance is spread over a larger quantity of product rather than because of a more effecient use of the feed she consumes.

REFERENCES

(1) Eckles and Reed 1912 A study of the cause of wide variation in milk production by dairy cows. Missouri Research Bulletin No. 2.

(2) Cooper, M. O., Bennett, C. M., and Church, L. M. 1917 A study of the cost of producing milk on four dairy farms located in Wisconsin, Michigan, Pennsylvania and North Carolina. U. S. D. A. Bulletin 501, pp. 35, fig. 8.

(3) Bronson, W. H. 1918 The cost of producing milk in Massachusetts. Mass. Agri. College Ext. Service Bulletin No. 19, pp. 20, fig. 1.

(4) North, C. E. 1919 Report of the Rochester Milk Survey, Committee of Public Safety, Common Council, Rochester, New York, pp. 227, fig. 3.

(5) Erf, O. 1914 Cost of milk production. Cream and Milk Plant Monthly, ii, no. 10, pp. 11.

(6) Thompson, A. L. 1915 Cost of producing milk on 174 farms in Delaware County. New York, Cornell Station Bulletin No. 364, pp. 109–179.

(7) Ross, H. A., Hall, H. F., and Rhode, C. S. 1923 The feed cost of milk and fat production as related to yields. Illinois Station Bulletin No. 244, pp. 552–573, fig. 3, Abs. Ed. pp. 4.

(8) Henry and Morrison 1922 Feeds and Feeding. Eighteenth Edition, pp. 349–350, paragraph 358.

(9) Converse, H. T. 1926 The effect on milk production of feeding more than the Haecker, Eckles and Savage requirements. Jour. Dairy Sci., ix, no. 4, 338–406 (fig. 3).

(10) Marshall, F. R. 1912 Relation between yields of milk and yields of fat in dairy cattle. American Breeders Magazine, May 3, ii, 148–149.

(11) Yule, G. Undy 1922 An Introduction to the Theory of Statistics, Sixth Edition.

SOME OBSERVATIONS ON THE BASIC VISCOSITY OF ICE CREAM MIXES*

J. C. HENING

New York Agricultural Experiment Station, Geneva, New York

In recent years the maximum viscosity of an ice cream mix has been termed apparent viscosity, and that viscosity which has been obtained by thorough agitation in mechanical devices, basic viscosity. Although this viscosity termed basic viscosity is the lowest viscosity attainable in a given ice cream mix under the conditions of agitation used, the question arises as to whether or not it is a true basic viscosity of the mix.

Some observations of fat clumps in thoroughly agitated ice cream mixes led to a further study of this problem. These studies tend to show that mechanical agitation, with air excluded, only partially breaks up the fat clumping in homogenized mixes and that these mixes have not attained their lowest possible viscosity for a given size of fat globule with this fat clumping present.

The clumping of fat globules in milk, cream and ice cream mixes has been shown by many investigators to increase the viscosity of these products. Likewise, two-stage homogenization of ice cream mixes has been shown to decrease fat clumping and reduce the viscosity of mixes.

Bateman and Sharp (1928) have published two important papers on the plasticity of milk and its products. They showed that milk, cream, and ice cream mixes were plastic and that even skimmilk was slightly plastic. They pointed out that since cream and ice cream mixes exhibit plasticity it is difficult to correlate single viscosity determinations with their other properties. They showed that the repeated running of a sample of whole milk through the capillary tube reduced its viscosity and a microscopic study of this milk showed that this reduction in viscosity

---

* Received for publication June 30, 1930.

was related to the breaking up of the fat clusters. In a similar manner they found that the repeated running of skimmilk through the capillary tube did not reduce its viscosity.

Several noteworthy papers on basic viscosity as related to ice cream mixes have been published from the Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture. Leighton and Williams (1927) showed that two kinds of viscosity exist in an ice cream mix, namely, the apparent viscosity or that present after aging the mix without agitation and the viscosity which remains after the mix has been thoroughly agitated, which they termed basic viscosity. They agitated their mixes in a freezer with air excluded for a period of time which gave the maximum reduction in viscosity. For example, a mix containing 36.3 per cent total solids after one hour agitation reached a constant minimum viscosity which could not be reduced by continued agitation. In a later paper Leighton and Kurtz (1929) showed that these agitated mixes did not have plastic properties.

EXPERIMENTAL METHODS

Ice cream mixes for these experiments were standardized to contain 12 per cent fat, 10 per cent serum solids and 14 per cent sugar. They were pasteurized at 62.7° to 65.5°C. (145° to 150°F.) and homogenized at the pasteurization temperature and 2000 pounds pressure in a Manton-Gaulin homogenizer of 60 gallons per hour capacity. They were cooled over a surface tubular cooler to 4.4°C. (40°F.).

Twenty per cent serum solid mixtures were prepared with skimmilk and skimmilk powder, and processed the same as the ice cream mixes.

The viscosity determinations were made with the MacMichael viscometer using the disc bob. The viscosity was measured at a temperature of 2.0 ± 0.3°C. No endeavor was made to secure separate values for plasticity and viscosity.

A motor driven stirrer as described by Whitaker (1929) was used to thoroughly agitate the mixes. The mixes were agitated for twenty minutes with the stirrer, made of one-fourth inch wire screening, revolving at 500 to 600 revolutions per minute. The apparatus provided for the exclusion of air during agitation.

The fat globules were measured at a magnification of approximately 2000 diameters using an ocular micrometer disc standardized with the microscope so adjusted that each of the smallest marks represented 0.625 micron. One-half cubic centimeter of the mix to be examined was diluted with 100 cc. of distilled water and mounted as a hanging drop preparation.

EXPERIMENTAL RESULTS

Two series of ice cream mixes containing butter, skimmilk, skimmilk powder, and sugar; and butter, skimmilk powder, water, and sugar which were prepared to study the effect of aging on their properties have been included in these results. These mixes contained 0.56 per cent of a medium grade calf skin gelatin. In the first series viscosity determinations were made on the agitated and unagitated mixes. The results are given in table 1. As can be seen from table 1, the twenty minutes of agitation greatly reduced the viscosity of these very viscous mixes. The fat clumps, however, were only partially broken up by the agitation. This reduction in viscosity can be attributed to the breaking down of the gel structure and a partial splitting of the fat clumps.

TABLE 1

Viscosity in centipoises of mixes prepared with butter, skimmilk, skimmilk powder, sugar and gelatin homogenized at 2,000 pounds

MIX	APPARENT			AGITATED BASIC		
	0 hours aged	4 hours aged	20 hours aged	0 hours aged	4 hours aged	20 hours aged
1	3,000	3,930	5,360	182	174	249
2	2,020	4,860	5,830	158	163	242
3	227	1,270	1,880	108	120	137

In the next series of butter mixes a portion of the mix which had been homogenized at 2000 pounds pressure was run through the homogenizer four times at 200 pounds pressure to eliminate the fat globule clumps as much as possible. This low pressure was chosen because it broke up fat clusters and it was assumed that such a low pressure would not alter other properties of the mix to any extent. There was, however, a slight difference in the size of individual fat globules in the 2000 and 200 pounds pressure mixes. Seven hundred individual globules in the 2000 pounds pressure mixes averaged 1.61 microns and 1000 individual globules in the 2000 and 200 pounds pressure mixes measured 1.40 microns. Viscosity determinations were made on these mixes as before.

TABLE 2

Viscosity in centipoises of mixes prepared with butter, skimmilk powder, sugar, water and gelatin

MIX	HOMOGENIZATION PRESSURE	APPARENT			AGITATED BASIC		
		0 hours aged	4 hours aged	20 hours aged	0 hours aged	4 hours aged	20 hours aged
1	2,000	1,270	5,830	7,500	139	129	125
	200*	129	1,010	1,050	68	98	120
2	2,000	640	1,760	3,680	96	87	113
	200*	106	880	1,310	65	86	118
3	2,000	221	1,830	2,800	60	105	99
	200*	77	680	960	51	70	74
4	2,000	1,520	3,600	4,870	103	133	179
	200*	55	560	930	45	57	83

* These mixes were homogenized at 2,000 pounds pressure and then four times at 200 pounds.

As is shown in table 2, this treatment greatly reduced the viscosity of these mixes in comparison with the portions of the mixes homogenized at 2000 pounds pressure. Also the viscosity of these mixes when agitated was less than the agitated mixes homogenized at 2000 pounds pressure. The presence of gelatin in these mixes introduced another factor which influenced their viscosity. An examination of these mixes under the microscope showed a direct relationship between the size of fat clusters and viscosity. Re-homogenization of the mix at 200 pounds pressure greatly reduced the original viscosity of the mix due to the splitting of fat clumps. The average size of fat clumps in mixes homogenized at 2000 pounds pressure was 7.55 by 4.11 microns and in the mixes re-homogenized at 200 pounds pressure 2.31 by 1.75 microns.

TABLE 3

Size of fat clumps in microns of mixes prepared with cream, skimmilk, skimmilk powder and sugar

MIX	HOMOGENIZATION PRESSURE	SIZE OF FAT GLOBULE CLUMPS	
		Apparent	Agitated basic
1	2,000	5 13 x 2 61	4.20 x 2.35
	200*	2 06 x 1 10	2 18 x 1 20
2	2,000	5 29 x 2 84	3 71 x 1 77
	200*	2.14 x 1 15	2 06 x 1 11
3	2,000	5 25 x 2 94	3 48 x 1 91
	200*	1.81 x 0 99	1 88 x 1 05

* These mixes were homogenized at 2,000 pounds pressure and then four times at 200 pounds.

TABLE 4

Viscosity in centipoises of mixes prepared with cream, skimmilk, skimmilk powder and sugar

MIX	HOMOGENIZATION PRESSURE	APPARENT		AGITATED BASIC	
		0 hours aged	20 hours aged	0 hours aged	20 hours aged
1	2,000	76 0	93 0	36 0	36 0
	200*	18.0	20 0	18 0	19.0
2	2,000		790 0	55 0	50 8
	200*		22 0		20.5
3	2,000	329 0	323.0	48.5	54 8
	200*	23 0	23 0	21.0	23.0

* These mixes were homogenized at 2,000 pounds pressure and then four times at 200 pounds pressure.

Another series of mixes standardized as before, were prepared without gelatin. Thirty per cent cream supplied the fat for these mixes in place of butter. These mixes were processed and viscosity determinations were made the same as in previous experiments. One hundred fat clusters and individual globules found in the same fields were measured in each of these mixes before and after agitation. The results of these experiments are given in tables 3 and 4. The fat clumps of the mixes homogenized at 2000 pounds pressure were twice as large as the fat clumps found in these same mixes, after running them through the homogenizer four times at 200 pounds pressure. The viscosites of the former mixes were 4, 36 and 14 times the viscosities of the latter. Agitation reduced the viscosity of the mixes homogenized at 2000 pounds pressure to one-half, one-fifteenth and one-sixth of the original viscosity and cut the size of the clumps by approximately one miron from each dimension. Agitation of the mixes run through the homogenizer at 200 pounds pressure did not reduce their viscosity or alter the size of the fat clusters. The mixes homogenized at 2000 pounds pressure had a much smaller number of individual fat globules in proportion to clumps than when they were homogenized at 200 pounds pressure. The average size of 2000 individual globules was 1.47 microns. The agitated 2000 pounds pressure mixes were twice as viscous and contained fat clumps nearly twice as large as the agitated 200 pounds pressure mixes.

In a previous paper by the author (1928) measurements were made of a large number of fat globule clumps in ice cream mixes before and after freezing. The average size of clumps in mixes before freezing was 5.89 by 3.51 microns and after freezing 4.31 by 3.03. These mixes were homogenized with the single-stage valve. These results show the ineffectiveness of agitation in destroying fat globule clumps formed in homogenized mixes. It was also shown that agitation during freezing reduced the size of fat globule clumps to a greater extent in some mixes than in others.

The effect of agitation on mixtures with little or no clumping should give further information on the relation of clumps to changes in viscosity of agitated mixtures. Two different lots of milk containing 5.5 per cent fat were pasteurized at 65.5°C. (150°F.) and homogenized at 2000 pounds pressure at the pasteurization temperature. The clumps of fat globules in this milk averaged 2.5 by 1.31 microns before agitation and after agitation 2.31 by 1.25 microns. The average viscosity of the two lots of milk at 0, 4, and 24 hour aging periods is as follows: 6.5, 6.25 and 6.00 centipoises. The viscosity of the agitated milk for the same periods is as follows: 6.66, 6.62 and 6.37 centipoises. There was practically no difference in the size of the clumps in the milk or the viscosity of the milk in either case.

An ice cream mix containing 10 per cent fat and 11 per cent serum solids was prepared as before except that it was homogenized with the two-stage valve at pressures of 3000 pounds on the first stage and 2200 on the second stage. Fourteen per cent sugar was added after homogenization. Fat globule clumping was almost eliminated but there were a few very small clumps present. The viscosity of this mix before agitation was 18.4 centipoises and 18.0 after agitation.

TABLE 5

Viscosity in centipoises of 20 per cent serum solid mixes prepared with skimmilk and skimmilk powder

MIX	TREATMENT	APPARENT			AGITATED BASIC		
		0 hours aged	4 hours aged	20 hours aged	0 hours aged	4 hours aged	20 hours aged
1	Unhomogenized						
	Homogenized	10.9	11.9	14.0	10.9	12.5	14.0
2	Unhomogenized	11.0	13.0	15.0	10.0	12.8	14.9
	Homogenized	10.5	14.0	15.5	11.9	11.9	14.9
3	Unhomogenized	14.0	14.0	16.0	16.5	15.5	16.0
	Homogenized	11.0	14.0	14.5	13.3	14.8	16.0
4	Unhomogenized	12.0	13.0	14.5		16.2	15.0
	Homogenized	11.5	10.5	12.0	12.5	12.2	12.2

In another series of experiments skimmilk was standardized with skimmilk powder to contain 20 per cent serum solids without milk fat. These mixtures were pasteurized at 65.5°C. (150°F.) and half of each mix was homogenized at 2000 pounds pressure. Viscosity determinations were made on unhomogenized and homogenized samples both with and without agitation. The results are given in table 5. In some cases due to the agitating device not working properly foam occurred on some of the agitated 20 per cent skimmilk and skimmilk powder mixtures. These agitated samples increased slightly in viscosity likely due to the incorporation of a small amount of air. In two cases the agitated sample was slightly less viscous than the unagitated sample. In all other cases the agitated samples were practically the same or higher in viscosity than the unagitated samples. The serum solids mixes without fat could not be decreased in viscosity with agitation.

SUMMARY

These results tend to show that a particular method of mechanical agitation of ice cream mixes reduces their viscosity by a partial splitting of fat clumps, to a constant low viscosity, attainable under the conditions of agitation, but the size of clumps and the viscosity can be reduced still further by more vigorous mechanical treatment as when the mix is subsequently run through the homogenizer at low pressure.

The presence of clumps in ice cream mixes which have been agitated for basic viscosity determinations is a point which has apparently not been given serious consideration. Different conditions of agitation may vary in their effectiveness in splitting clumps and introduce a variable factor in the magnitude of the basic viscosity secured.

Basic viscosity, as the term is now used for ice cream mixes, applies to a value secured under specified conditions and it is not a correct minimum value from the viewpoint of the lowest viscosity that might be present without fat clusters.

REFERENCES

Bateman, G. M., and Sharp, P. F. 1928 A study of the apparent viscosity of milk as influenced by some physical factors. Jour. Agr. Res., xxxvi, 647–674.

Bateman, G. M., and Sharp, P. F. 1928 Some observations on the consistency of cream and ice cream mixtures. Jour. Dairy Sci., xi, 380–396.

LEIGHTON, A., AND WILLIAMS, O. E. 1927 The basic viscosity of ice cream mixes. Jour. Phys. Chem., xxxi, 596.

LEIGHTON, A., AND KURTZ, F. E 1929 The basic viscosity and plasticity of ice cream mixes. Jour. Phys. Chem., xxxiii, 1485–1488.

HENING, J. C. 1928 Effect of homogenizing ice cream mixes before and after the addition of gelatin or sugar and before and after condensing. Jour. Dairy Sci., xi, 307.

WHITAKER, R. 1929 A device for reducing an ice cream mix to its basic viscosity. Jour. Dairy Sci., xii, 285–287.

A TEST FOR THE DETECTION OF MILK UNSTABLE TO HEAT*

GUY A. RAMSDELL, WM. T. JOHNSON, JR., AND FRED R. EVANS

Bureau of Dairy Industry, U. S. Department of Agriculture

INTRODUCTION

An accurate and rapid method for the detection of milk unstable to heat should be of value to the evaporated milk industry. Unfortunately, the literature has failed as yet to reveal such a test. Although a great deal of work has been done in ascertaining the relationships between heat stability and titratable acidity (5), true acidity expressed in pH values (5) (3), concentration of mineral salts (5) (3), and reaction towards alcohol of the whole milk samples (4) (2) (1), not any of these comparisons have been found to be sufficiently definite for advantageous use in grading milk for sterilization purposes. In fact, no test is known wherein the reactions of the test duplicate the reactions produced when milk is heated to sterilization temperatures and, consequently, serve as a criterion of its heat stability. However, a test that will give a truer analysis of the fitness of milk for condensing than any of the existing methods should be of value, and such a test is described in this paper.

The test was developed from the results obtained when making comparisons between heat stability values of the concentrated products and the precipitating actions of numerous acids and salts on the whole milk samples, when such mixtures were heated to a temperature of boiling water. Mono basic potassium phosphate was finally selected, since it was found to be the most satisfactory precipitating agent. The actual coagulation was brought about through the combined action of the added acid salt and heat.

This paper records a description of the test, results obtained, and a discussion of its limitations. Data are also presented show-

---

* Received for publication June 19, 1930.

ing the relationship between heat stability and minimum concentration of phosphate solution necessary to produce initial coagulation of the milk when added to milk in the manner described in the test.

EXPERIMENTAL

The milk was obtained from herds representing Holstein, Jersey, and Guernsey stock. However, some samples were from registered cattle all of one breed. The samples were collected during the spring, fall, and winter months.

The heat stabilities were determined by forewarming the milk to 95°C. for 10 minutes, concentrating in a partial vacuum, standardizing to 18 per cent solids not fat, sealing in tin cans, and finally sterilizing in a pilot sterilizer. Fifteen minutes were taken to bring the milk to the sterilization temperature of 121°C. Whole milk was always used so that the concentrated product contained approximately 6 to 12 per cent fat. Small changes in fat content had no appreciable effect on the phosphate test.

Throughout this work, the phosphate test was conducted in the following manner:

Solution used: 0.5 M KH_2PO_4 (68.1 grams of mono basic potassium phosphate were dissolved in water and the solution diluted to one liter).

Two cubic centimeter samples of milk were transferred to Pyrex test tubes of approximately 20 cc. capacity, and 0.20 cc. of the phosphate solution added accurately to each tube by means of a 1 cc. pipette, calibrated in 0.1 cc. divisions. The pipette was of small bore, so that the divisions were far enough apart to permit measurements within an accuracy of 0.01 cc.

The contents of the tubes were then thoroughly mixed and the tubes immersed in boiling water for 5 minutes. They were then removed, cooled, and the mixture examined for the presence of a curd. Any coagulation indicated that the concentrated product was of low heat stability.

The test could be made applicable to factory routine if larger amounts of both milk and phosphate solution were used. A satisfactory proportion would be 10 cc. of milk and 1 cc. of the phosphate solution. This would permit the use of a burette for dispensing the phosphate solution. Likewise, the milk could be measured out very rapidly by means of a 10 cc. dipper. With reasonable caution, 10 cc. plus or minus 0.1 cc. of milk can be obtained by this means.

The use of these larger amounts of milk and phosphate solution would obviously reduce errors in measuring, and since there is a fairly definite relationship between the amount of milk and concentration of phosphate solution to produce a curd, results obtained by both methods on the same sample of milk should be comparable. However, when using these larger amounts, care must be taken to have the mixture immersed below the top of the boiling water to insure the necessary heating.

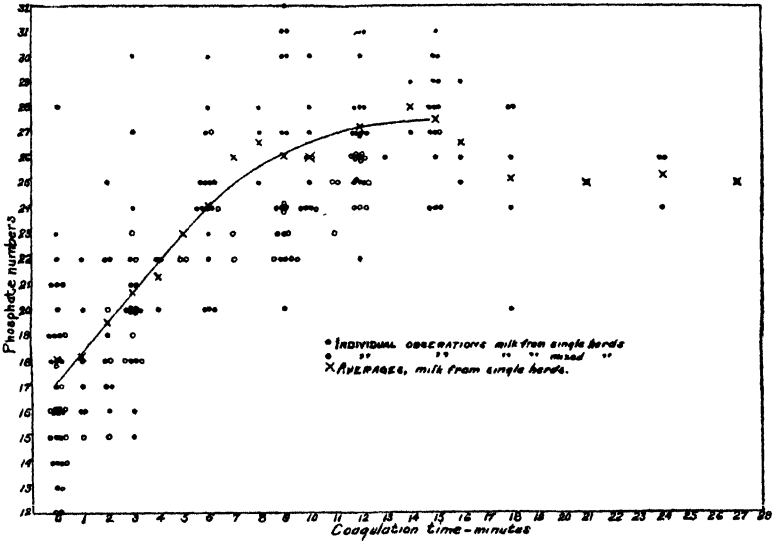


FIG. 1. RELATION BETWEEN PHOSPHATE NUMBERS AND COAGULATION TIME

A series of comparisons were first made between the actual concentration of phosphate solution necessary to produce initial coagulation of whole milk samples and the heat stability of the respective evaporated products. The object of this inquiry was to determine the amount of solution to use in the test. The procedure was similar to that described in the phosphate test, only, in addition, increasing amounts of the solution were added to 2 cc. portions of each sample until the amount within 0.02 cc. was found that would just produce initial coagulation. Approximately 150 individual comparisons were made and the results are plotted in figure 1. A summary of these observations is shown in table 1. The number of fractional cubic centimeters necessary to curdle the milk, multiplied by 100, is designated as the phosphate number. These values are listed in the second column of this table.

TABLE 1

*Comparison between coagulation time and average phosphate numbers of milks of similar heat stability values**

COAGULATION TIME	PHOSPHATE NUMBER (AVERAGE)	NUMBER OF TESTS
minutes		
0	18.1	25
1	18.2	6
2	19.5	8
3	20.7	16
4	21.3	3
5	23.0	1
6	24.1	16
7	26.0	1
8	26.6	3
9	26.0	17
10	26.0	8
12	27.2	17
13	26.0	1
14	28.0	2
15	27.5	14
16	26.6	3
18	25.2	5
21	25.0	1
24	25.3	3
27	25.0	1
30	28.0	1
33	27.0	1
36	28.0	1

* Single herd milk samples.

The heat stabilities of the milks varied from 0 to 36 minutes and the phosphate numbers from 12 to 32, which represents a considerable range of milks both as to heat stability and phosphate resistance.

As is observed upon inspection of figure 1, there is a striking agreement in the heat stability of those milks that have a phosphate number of 20 or less; over 86 per cent of them coagulated in the sterilizer in less than 4 minutes. They were milks of distinctly poor heat resistance. This relationship is even more pronounced in composite samples from mixed herds. All of the composite samples that had a phosphate number of 20 or less coagulated before 4 minutes. With increasing phosphate numbers there is at first a general trend towards an increase in heat stability values. However, this relationship does not exist in milks of high phosphate numbers. A high phosphate number may not necessarily indicate a high heat resistant milk. In fact, samples with a phosphate number of 24 have coagulated in anywhere from 3 to 24 minutes under sterilization, while one sample with a phosphate number of 32 coagulated in only 9 minutes.

The relationship between coagulation time and average phosphate numbers of milks of the same heat resistance (listed in table 1) is also plotted in figure 1. A fairly definite curve can be traced up to a limiting value of 12 minutes in coagulation time. Beyond this there is little or no correlation.

Table 2 shows the results obtained when applying the phosphate test to cull out the unstable milks. The procedure was to separate from 20 to 120 samples representing as many herds selected each day into three composite batches, A, B, and C. Composite A consisted of an aliquot of the samples that coagulated with the phosphates test, composite B an aliquot of the samples that did not coagulate with the phosphate test, and composite C an aliquot of all the sample selected each day.

The phosphate numbers of these composites were then determined, as well as the heat stability of their forewarmed and concentrated products. The stabilities of the A milks were in every run less than the C milks, and no batch of A milks held up in the sterilizer longer than 3 minutes, while approximately 70 per cent

TABLE 2

Stability of composite samples of milk separated on basis of phosphate test

NUMBER	COMPOSITE	COMPOSITION OF COMPOSITES		COAGULATION TIME	CHANGE IN STABILITY FROM TOTAL COMPOSITE (C)
		A	B		
		per cent	*per cent*	*minutes*	*minutes*
1	A*	100	0	0	5−†
	B	0	100	12	7+
	C	32	68	5	
2	A	100	0	0	5−
	B	0	100	6	1+
	C	21	79	5	
3	A	100	0	1	6−
	B	0	100	9	2+
	C	33	66	7	
4	A	100	0	0	9−
	B	0	100	12	3+
	C	15	85	9	
5	A	100	0	0	9−
	B	0	100	12	3+
	C	27	73	9	
6	A	100	0	3	9−
	B	0	100	15	3+
	C	27	73	12	
7	A	100	0	0	10−
	B	0	100	12	2+
	C	20	80	10	
8	A	100	0	0	2−
	B	0	100	14	12+
	C	24	76	2	
9	A	100	0	0	9−
	B	0	100	12	3+
	C	26	74	9	
10	A	100	0	0	9−
	B	0	100	12	3+
	C	20	80	9	
11	A	100	0	0	1−
	B	0	100	5	4+
	C	36	64	1	

TABLE 2—*concluded*

NUMBER	COMPOSITE	COMPOSITION OF COMPOSITES		COAGULATION TIME	CHANGE IN STABILITY FROM TOTAL COMPOSITE (C)
		A	B		
		per cent	*per cent*	*minutes*	*minutes*
12	A	100	0	3	6−
	B	0	100	12	3+
	C	34	66	9	
13	A	100	0	0	9−
	B	0	100	12	3+
	C	27	73	9	
14	A	100	0	0	9−
	B	0	100	12	3+
	C	40	60	9	
15	A	100	0	0	3−
	B	0	100	6	3+
	C	27	63	3	
16	A	100	0	0	7−
	B	0	100	11	4+
	C	17	83	7	
17	A	100	0	2	9−
	B	0	100	11	0
	C	25	75	11	
18	A	100	0	3	9−
	B	0	100	12	0
	C	16	84	12	
19	A	100	0	2	8−
	B	0	100	10	0
	C	45	55	10	

* A Composite of milks that coagulated with the phosphate test.
B Composite of milks that did not coagulate with the phosphate test.
C Composite of all milks selected each day.

† − Decrease in stability.
+ Increase in stability.

coagulated at 0 minutes, or before the sterilization temperature was attained. It is observed that the composites made up of samples that coagulated with the phosphate test were invariably poor heat resistant milks. Hence these results afford excellent evidence of the value of the phosphate test to detect milks of this class. The increase in stability of the B milks over that of the A milks ranged from 5 to 14 minutes. In runs Nos. 17, 18, and 19, no improvement was noted in B milks over that of the total batch or C milks. However, the stability of B milks has never been less than the C milks.

These observations are shown in figure 2. The area above the C line represents an increase in stability over C milks, and the area below a decrease in stability.

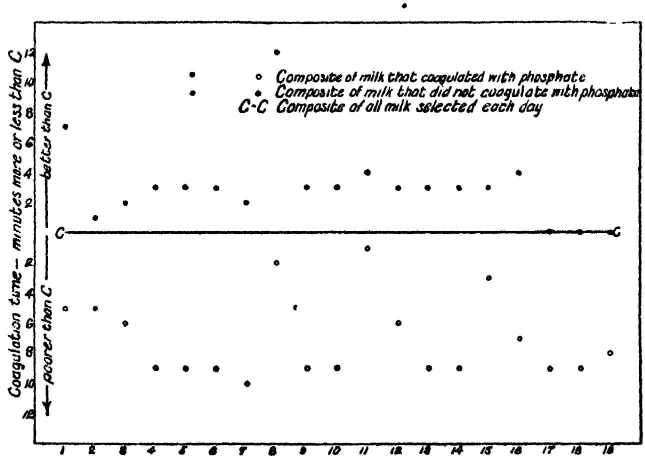


FIG. 2. COMPARATIVE STABILITY OF COMPOSITE SAMPLES OF MILK SEPARATED ON THE BASIS OF REACTION TO THE PHOSPHATE TEST

The composites where no improvement was made by separating the unstable milks (A milks) gave nearly similar phosphate numbers to that of the C milks, with the exception of sample 19, where the phosphate number of batch C was 24 and batch B, 29.

A comparison between pH values and phosphate numbers failed to show any consistent relationship. Samples with similar phosphate numbers have had pH values anywhere from 6.40 to 6.70. Only in those samples where the H-ion concentration is distinctly abnormal, no doubt due to bacterial growth, are the phosphate numbers of the same order.

Phosphate tests were made intermittently during a period of six months on milk samples from approximately 100 different herds. The purpose was to determine the variation, if any, in the reaction to the phosphate test in milk samples from the same herd collected at daily or weekly intervals. It was found that in a large majority of the herds the milk was fairly constant from day to day in its reaction to the phosphate test. The fact that the milk was phosphate positive on one day was fairly good assurance that it would be phosphate positive the day following. Milk from several herds was consistently phosphate positive for over a period of three months, while milk from other herds was phosphate negative during two or three months at a time. However, milk from a few herds (representing about 5 per cent of all studied) would fluctuate some from day to day in its reaction to the test, and others over longer intervals. These samples had phosphate numbers that varied between 20 and 22. While they were not consistent in their reaction to the test, their actual daily variation in phosphate resistance was small. Milk from one particular herd in which a study was made for over a year, was always of good quality in respect to its heat stability and the phosphate numbers were never less than 26. This herd consisted of registered Holstein cattle of very large frame. The cows were milked three times daily. Another herd gave milk throughout the year of low heat stability and of phosphate numbers consistently below 22. In general, milk from the same herd that reacts phosphate positive or phosphate negative on one day, is quite apt to react the same the day following and often one week later. However, it is suggested that tests should be made at least twice a month in order to maintain an adequate check.

Addition of disodium phosphate, to the milk that coagulated in the phosphate test always resulted in an improved product. On the other hand, milks of high phosphate numbers were generally reduced in their resistance to heat when treated with the disodium phosphate. Work is now in progress to determine what relationship does exist, if any, between phosphate numbers and amount of disodium phosphate to add for optimum stability.

DISCUSSION

A study of the results presented shows that milk coagulated in the phosphate test is of low heat stability. Furthermore, composites made up of samples of milk of this class are also of low heat stability. Likewise, elimination of the milks that respond to the phosphate test from the total supply, often results in obtaining a more stable product, and an increase in stability is invariably produced when the percentage of these milks represent over 50 per cent of the total milk under investigation. However, the heat stabilities of composites are not always increased by eliminating the samples that respond to the test, at least when the percentage of poor milks is small in comparison to the total amount. In fact, it was found that one batch was not improved at all by eliminating the poor stability milks. The poor milks comprised 45 per cent of the total amount studied on this date. Then again, another batch was increased 3 minutes in stability

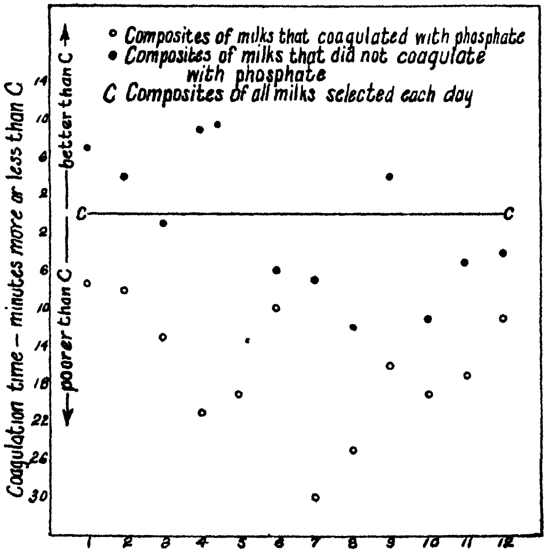


FIG. 3. COMPARATIVE STABILITY OF COMPOSITE SAMPLES OF CONDENSERY MILK SEPARATED ON BASIS OF PHOSPHATE TEST

TABLE 3

*Stability of composite samples of milk separated on basis of phosphate test, when using 10 cc. of milk and 1 cc. of phosphate solution in the test**

NUMBER	COMPOSITE	COMPOSITION OF COMPOSITES		COAGULATION TIME	CHANGE IN STABILITY FROM TOTAL COMPOSITE (C)
		A	B		
		per cent	*per cent*	*minutes*	*minutes*
1	A†	100	0	8	7−‡
	B	0	100	22	7+
	C	40	60	15	
2	A	100	0	2	8−
	B	0	100	14	4+
	C	33	66	10	
3	A	100	0	2	13−
	B	0	100	14	1−
	C	16	84	15	
4	A	100	0	1	21−
	B	0	100	31	9+
	C	8	92	22	
5	A	100	0	1	19−
	B	0	100	Lost	
	C	8	92	20	
6	A	100	0	5	10−
	B	0	100	9	6−
	C	37	63	15	
7	A	100	0	7	30−
	B	0	100	30	7−
	C	37	63	37	
8	A	100	0	8	25−
	B	0	100	21	12−
	C	33	67	33	
9	A	100	0	9	16−
	B	0	100	29	4+
	C	33	67	25	
10	A	100	0	11	19−
	B	0	100	19	11−
	C	33	67	30	

TABLE 3—*concluded*

NUMBER	COMPOSITE	COMPOSITION OF COMPOSITES		COAGULATION TIME	CHANGE IN STABILITY FROM TOTAL COMPOSITE (C)
		A	B		
		per cent	*per cent*	*minutes*	*minutes*
11	A	100	0	8	17−
	B	0	100	20	5−
	C	16	84	25	
12	A	100	0	18	11−
	B	0	100	25	4−
	C	25	75	29	

* Results obtained by B. H. Webb, in a condensery in California.

† A Composite of milks that coagulated with phosphate test.
B Composite of milks that did not coagulate with phosphate test.
C Composite of all milks selected each day.

‡ − Decrease in stability.
+ Increase in stability.

by separating out the poor milks which represented only 15 per cent of the total amount (by volume) used that day.

Webb[1] found somewhat similar results when applying the test at a condensery in California. His results are tabulated in table 3, and comparisons of his A and B composites with his C composites are shown in figure 3. The milk arriving at the condensery at that time was very stable and the rejection of the poor milks or the use of a neutralizer was seldom necessary. However, the A composites were always of lower heat stability than either the C or B composites, which is in accord with our findings. On several days Webb found that certain percentage mixtures of his A and B composites gave a more stable product than his B composites alone. In this connection, that is when mixtures of the A and B milks are made, a determination of the phosphate numbers of such mixtures will often aid in approximating their resultant stabilities. By this means, mixtures of the requisite heat stability may be obtained.

In view of the fact that elimination of poor stability milk does not always result in producing a resultant milk of higher stability, use of the test should be governed by the results obtained and consideration should be given to the type of milk arriving at the condensery. Its real purpose is to aid in picking out those milks that impair the stability of the total composite during the seasons when sterilization is giving trouble, and not to be used as a quality test of sanitary milk. The amount of solution to use in the test is likewise variable, and should be governed by the type of milk under examination. When the elimination of milk that coagulates when 1 cc. of the phosphate solution is treated with 10 cc. of milk does not produce an increased stability in the remaining batch, the use of lesser amounts might. The appropriate amount can be ascertained by trial only.

---

[1] From an unpublished report made by Byron H. Webb, of this laboratory.

SUMMARY AND CONCLUSIONS

1. A test has been developed for the detection of milk unstable to heat. The test is rapid and made applicable to factory routine.
2. Milk that coagulates in the test is invariably of low heat resistance. Composites of this class of milk are also of low heat stability.
3. In grading milk on the basis of the phosphate test, the elimination of the phosphate positive milk often results in obtaining a milk of higher stability towards heat.
4. Phosphate number is defined.
5. Relation between phosphate number and coagulation time is shown.
6. There is no apparent relationship between pH and phosphate number.
7. Single herds are reasonably constant in their phosphate number.
8. This test has been tried on a commercial scale in a condensery in California.

REFERENCES

(1) Ayers, S. H., and Johnson, W. T., Jr. 1915. The alcohol test in relation to milk. U. S. Dept. Agr., Bul. 202, 35 p., illus.

(2) Dahlberg, A. O., and Garner, H. S. 1921. The alcohol test as a means of determining quality of milk for condenseries. U. S. Dept. Agr., Bul. 944, 13 p., illus.

(3) Rogers, L. A., Deysher, E. F., and Evans, F. R. 1921. The relation of acidity to the coagulation temperature of evaporated milk. Jour. Dairy Sci., iv, *294–309*, illus.

(4) Sommer, H. H., and Binney, T. H. 1923. A study of the factors that influence the coagulation of milk in the alcohol test. Jour. Dairy Sci., vi, 176–197, graphs.

(5) Sommer, H. H., and Hart, E. B. 1926. The heat coagulation of evaporated milk. Wis. Agr. Expt. Sta. Research Bul. 67, 47 p., illus.

SPEED OF DASHER AND SCRAPER AS AFFECTING THE QUALITY OF ICE CREAM AND SHERBET*

E. L. REICHART

Department of Dairy Husbandry, University of Nebraska, Lincoln

INTRODUCTION

The texture of ice cream is largely determined by the structure of the ice crystals constituting the body or bulk of ice cream and the air spaces separating these crystals. The smoothness of ice depends upon controlling the crystal and air cell formation. Factors entering into the control of crystal and air cell structure may be classified into two general groups: First, ingredient factors (2, 5); and second, processing factors. It is with a phase of this second group of factors that these experiments deal.

It is well known that freezing conditions have a marked influence on the body and texture of the finished ice cream (1, 4). Of these, brine temperatures, sharpness of scraper blades, and the operation of the movable parts of the freezer are especially important (3). The major part of these trials deals with the operation of the scraper and dasher.

EQUIPMENT AND METHODS

The freezer used was a forty-quart Fort Atkinson, which had been rebuilt and equipped with several sets of different size gears. It was so arranged that the dasher could be operated independently from the scraper and a motor was attached to drive it independent of the scraper. A two-way switch was attached to the motor driving the dasher to provide for the operation of the dasher in either direction, i.e., opposite to the direction of the scraper as is normal, and in the same direction. By not turning on the dasher motor, it was also possible to have the dasher idling, being propelled in neither direction, but offering some resistance to the freezing mix due to its presence as an obstacle.

* Received for publication July 18, 1930. Submitted to the JOURNAL OF DAIRY SCIENCE as Contribution No. 94 from the Nebraska Experiment Station.

The ice cream mix used in these trials contained 14 per cent fat, 10 per cent serum solids, 14 per cent sugar and 0.25 per cent gelatin, and was made from fresh cream, skim-milk, and condensed skim-milk (30 per cent serum solids). It was pasteurized at 145°F. for 30 minutes and homogenized with a two stage Colony homogenizer at 2000 and 1000 pounds pressure, cooled immediately to between 40 and 50°F. and then frozen with various times of aging.

TABLE 1

Influence of speed of dasher upon freezing ice cream

Date of experiment: October 2, 1929. Mix composition: Fat, 14 per cent; serum solids, 10 per cent; sugar, 14 per cent; gelatin, 0.25 per cent. Hardening room temperature: −5°F.

SAMPLE	FREEZING CONDITIONS	GEAR RATIO DASHER	DASHER SPEED	OVERRUN AT MINUTES INDICATED						BRINE TEMPERATURE	AGE OF MIX
				3	5	7	9	11	13		
			r p m.							*°F*	*hours*
1	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	45	55*	72	85	100		−15	40
2	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	60	70*	70	75	85	100	−15	40
3	Scraper normal (295 r.p.m.) dasher low speed	94–14	163	80	70*	72	81	90	95	−13	42
4	Scraper normal (295 r.p.m.) dasher low speed	94–14	163	70	65*	70	81	94	100	−13	42
5	Scraper normal (295 r.p.m.) dasher medium speed	94–16	200	60	70*	70	79	95	110	−15	72
6	Scraper normal (295 r.p.m.) dasher medium speed	94–16	200	50	59*	68	80	96	100	−15	72

* Time when brine was shut off.

Experimental work

Trial I. Variations of dasher speed. This trial consisted of three duplicate runs with the dasher operated at high, low, and medium speed, respectively. At high speed the dasher was turning 260 revolutions per minute; at medium speed (normal), 200; and at low speed, 163 revolutions per minute. The scraper was operated at normal speed (295 r.p.m.). Overrun readings were taken as indicated with the attempt made to freeze all mixed to 100 per cent overrun.

Samples from the above batches were placed in the hardening room, allowed to harden for 48 hours, and then examined for body and texture differences by four members of the department. It was their unanimous opinion that there were no appreciable differences in the texture or body of the six different samples as shown in table 1. Figures in the overrun column indicate that there was very little difference in the length of time necessary either to freeze the ice cream sufficiently or to incorporate 100 per cent overrun. These trials seemed to indicate that the variation of the dasher speed from 163 to as high as 260 revolutions per minute had no marked effect on the finished product, the freezing time or the incorporation of overrun in a mix of the above composition.

Trial II. It was thought however, that in freezing sherbets, especially where difficulty frequently was encountered in keeping down the overrun, that the speed of the dasher might be the cause of the difficulty. With this idea in mind, a series of trials with sherbets were planned and carried out as shown in table 2.

In table 2 are presented the results of 20 trials with sherbet. It is a well-known fact that it is easy to obtain a large overrun in freezing sherbet, while only a moderate overrun is desired. Experience had indicated that under ordinary circumstances an overrun of between 30 and 40 per cent gives most satisfactory results, because of the better body. The first 8 samples represent trials with the scraper operating at normal speed, while the dasher was operated at high speed. The desired overrun was obtained at between 7 and 9 minutes and after that the whipping incorporated too much air. The second set of trial samples, 9 to 16 inclusive, had the scraper operating at normal and the dasher operating at normal or medium. In these trials the desired overrun was obtained in the same time, but there was not the excessive whipping afterward. In the third set of trials, samples 17 to 24 inclusive, the scraper was operated at normal speed, and the dasher was operated at slow speed. Here the overrun was incorporated somewhat slower throughout the entire whip-

TABLE 2

Influence of dasher speed upon freezing sherbet

Date of experiment: October 8, 16, 30, November 4, 1929. Mix composition: Sugar, 27 per cent; serum solids, 0.032 per cent; stabilizer, 0.0068 per cent. Hardening room temperature: −5°F.

SAMPLE	FREEZING CONDITIONS	GEAR RATIO DASHER	DASHER SPEED	OVERRUN AT MINUTES INDICATED						BRINE TEMPERATURE
				5	7	9	11	13	15	
			r.p m.							*°F.*
1	Scraper normal (295 r.p m.) dasher high speed	94–21	260	15	25	35	70	110	140	−5
2	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	15	25	35	75	115	120	−5
3	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	14	28	36	70	110	130	−5
4	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	16	24	38	80	120	135	−5
5	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	12	22	30	65	100	120	−5
6	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	15	25	35	70	105	140	−5
7	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	18	28	40	75	115	160	−5
8	Scraper normal (295 r.p.m.) dasher high speed	94–21	260	15	25	35	70	110	120	−5
Average				15	25.2	35.5	71.8	110.6	133.1	−5
9	Scraper normal (295 r.p.m.) dasher medium	94–16	200	15	20	30	60	75	89	−5
10	Scraper normal (295 r.p.m.) dasher medium	94–16	200	15	23	35	58	80	95	−5
11	Scraper normal (295 r.p.m.) dasher medium	94–16	200	16	20	35	60	70	86	−4
12	Scraper normal (295 r.p.m.) dasher medium	94–16	200	15	25	40	50	80	94	−4
13	Scraper normal (295 r.p.m.) dasher medium	94–16	200	14	20	38	45	75	75	−4
14	Scraper normal (295 r.p.m.) dasher medium	94–16	200	16	24	36	52	85	88	−3
15	Scraper normal (295 r.p.m.) dasher medium	94–16	200	15	22	35	54	80	82	−3
16	Scraper normal (295 r.p.m.) dasher medium	94–16	200	18	28	40	50	75	80	−3
Average				15.5	22.7	36.1	53.6	77.5	86.1	−3.8

17	Scraper normal (295 r.p.m.) dasher slow	94–14	163	10	15	20	25	27	45	−5
18	Scraper normal (295 r.p.m.) dasher slow	94–14	163	10	15	28	30	35	60	−5
19	Scraper normal (295 r.p.m.) dasher slow	94–14	163	10	14	34	35	40	56	−5
20	Scraper normal (295 r.p.m.) dasher slow	94–14	163	8	15	27	32	42	50	−5
21	Scraper normal (295 r.p.m.) dasher slow speed	94–14	163	9	16	30	40	50	65	−5
22	Scraper normal (295 r.p.m.) dasher slow speed	94–14	163	10	14	25	28	38	48	−5
23	Scraper normal (295 r.p.m.) dasher slow speed	94–14	163	10	15	28	34	40	60	−4
24	Scraper normal (295 r.p.m.) dasher slow speed	94–14	163	10	14	24	38	44	65	−3
Average				9 6	14 7	27	32 7	39 5	56 1	−4 6
25	Scraper normal (295 r.p.m.) dasher idle			10	12	20	24	35	40	−4
26	Scraper normal (295 r.p.m.) dasher idle			10	14	25	27	32	30	−4
27	Scraper normal (295 r.p.m.) dasher idle			10	14	22	24	34	35	−4
28	Scraper normal (295 r.p.m.) dasher idle			10	14	23	24	30	32	−3
29	Scraper normal (295 r.p.m.) dasher idle			10	12	21	26	35	32	−4
30	Scraper normal (295 r p.m.) dasher idle			8	14	20	25	30	35	−3
31	Scraper normal (295 r.p.m.) dasher idle			8	12	24	28	30	35	−3
32	Scraper normal (295 r.p.m.) dasher idle			8	10	20	24	32	30	−3
Average				9 2	12 7	21 8	25 2	32 2	33 6	−3.5

ping period and the maximum obtained was much lower. The last series of trials, samples 25 to 32 inclusive—were run with the scraper normal and the dasher idle. In these trials the overrun was obtained slower than in the other trials and the maximum reached was less. As the mix is usually frozen sufficiently to draw in 9 minutes, it is apparent that operating the dasher at a low speed or idle, assists in keeping the overrun down to a desirable amount for sherbet. Where brine temperatures are not low enough to complete freezing by the time the desired overrun has been reached, the time necessary to complete freezing usually allows too much whipping unless the dasher is operated slower than normal. These trials raise the question of the desirability of having a variable speed dasher in brine freezers used for making sherbets.

Trial III. A study of table 1 indicates that the variations in the dasher speed had but little effect upon the rapidity of obtaining overrun or upon the body and texture of the finished ice cream. This suggested that the scraper speed might be of greater influence than the dasher speed in obtaining more rapid incorporation of overrun.

In order to determine the effect of the scraper as compared with the dasher upon the freezing of ice cream, a series of runs was made in which the dasher was operated at normal speed, while the scraper was operated at different speeds. The same composition mix was used as in the previous trials with ice cream. In table 3 the results obtained are presented in tabular form.

In table 3 samples 1 and 2; and 6, 7, and 8 were frozen with scraper and dasher operating at normal speeds. The desired overrun, 100 per cent was obtained in 13 minutes in each case. Samples 3, 4, and 5; and 9, 10, and 11 were frozen with dasher operating at normal speed, but with the scraper speeded up 35 revolutions per minute. In these runs the desired overrun was obtained in 9 minutes with 4 samples and in 11 minutes with 2 samples. There was a saving of from 2 to 4 minutes in freezing time. These samples were hardened and then scored by four members of the department and a slightly smoother body was observed in the samples frozen at a high scraper speed. The

TABLE 3

Influence of scraper speed upon freezing ice cream

Date of experiment: October 8, 1929. Mix composition: Fat, 14 per cent; serum solids, 10 per cent; sugar, 14 per cent; gelatin, 0.25 per cent. Hardening room temperature: −3°F.

SAMPLE	FREEZING CONDITIONS	GEAR RATIO SCRAPER	SCRAPER SPEED	OVERRUN AT MINUTES INDICATED						BRINE TEMPERATURE	AGE OF MIX
				3	5	7	9	11	13		
			r p m.							*°F.*	*hours*
1	Scraper normal, dasher normal (200 r.p m.)	83–21	295	60	70	70*	79	95	110	−15	72
2	Scraper normal, dasher normal (200 r p m)	83–21	295	50	59*	68	80	96	100	−15	72
3	Scraper fast, dasher normal (200 r.p m.)	53–15	330		58*	71	90	105		−12	72
4	Scraper fast, dasher normal (200 r.p m)	53–15	330	50	67*	96	101			−12	72
5	Scraper fast, dasher normal (200 r p.m)	53–15	330		43*	90	100			−12	72
6	Scraper normal, dasher normal (200 r p m)	83–21	295	61	70*	70	75	88	100	−10	72
7	Scraper normal, dasher normal (200 r p m)	83–21	295	55	65*	72	78	90	101	−10	72
8	Scraper normal, dasher normal (200 r.p m)	83–21	295	50	58*	68	80	95	100	−10	72
9	Scraper fast, dasher normal (200 r.p m)	53–15	330	46	60*	75	94	103		−10	72
10	Scraper fast, dasher normal (200 r.p.m)	53–15	330	52	68*	90	100			−10	72
11	Scraper fast, dasher normal (200 r.p m.)	53–15	330	51	72*	85	98			−10	72

* Time when brine was shut off

saving of time appears to be advantageous from a commercial standpoint, especially when there apparently is a slight improvement in body and texture. The operation of the scraper at a speed higher than normal tends however to create so much centrifugal force within the freezer that it is difficult to get the mix into the freezer from the hopper without discontinuing the operation of the scraper.

TABLE 4

Influence of scraper and dasher upon freezing ice cream

Date of experiment: October 16, 1929. Mix composition: Fat, 14 per cent; serum solids, 10 per cent; sugar, 14 per cent; gelatin, 0.25 per cent. Hardening room temperature: −5°F.

SAMPLE	FREEZING CONDITIONS	GEAR RATIO DASHER	DASHER SPEED	OVERRUN AT MINUTES INDICATED						BRINE TEMPERATURE	AGE OF MIX
				3	5	7	9	11	13		
			r.p m							*°F.*	*hours*
1	Scraper fast (330 r.p.m.) dasher idle				48*	70	87	94	99	−10	24
2	Scraper fast (330 r.p.m.) dasher idle				45*	60	84	93	98	−10	24
3	Scraper fast (330 r.p.m.) dasher idle				46*	72	83	95	98	−8	24
4	Scraper fast (330 r.p.m.) dasher idle				48*	74	86	95	100	−8	24
5	Scraper fast (330 r.p.m.) dasher same direction as scraper	94–16	200		51*	65	79	88	96	−8	28
6	Scraper fast (330 r.p.m.) dasher same direction as scraper	94–16	200		51*	65	82	95	99	−8	28
7	Scraper fast (330 r.p.m.) dasher same direction as scraper	94–16	200		50*	65	80	96	100	−8	28

* Time when brine was shut off.

Trial IV. In order to further check the influence of the dasher upon the freezing of ice cream, runs were made with the dasher either idling, or operating in the same direction as the scraper, at medium or normal speed, while the scraper was operated at high speed. The results are presented in table 4.

The time required to obtain satisfactory overrun was uniformly 13 minutes in these runs. This is the same time that was needed when the dasher speed was high, and the scraper normal, (table 1, sample 2, (sample 1 froze in 11 minutes); dasher speed low, scraper normal, table 1, samples 3 and 4; dasher speed normal and scraper normal, table 1, samples 5 and 6). This seems to indicate that within the speed limits used, the speed of the dasher has no marked influence upon the time necessary to incorporate desired overrun. The mix used in this trial was identical in composition with that used in previous trials and the resulting ice cream after hardening was pronounced no different in texture and body, by four members of the department, from the ice cream produced in the other trials.

SUMMARY

1. The speed of the dasher (200 r.p.m. normal; 163 r.p.m. low; 260 r.p.m. high) has no appreciable effect upon the rapidity of freezing ice cream; upon the rapidity of incorporating 100 per cent overrun; or upon the body and texture of the finished ice cream, when made from fresh dairy products.
2. In freezing sherbets, where an overrun in excess of 30 to 40 per cent is undesirable, the reduction of the effect of the dasher by reducing its speed, by allowing it to idle, or by operating at normal speed in the same direction as the scraper, is useful in preventing the too rapid incorporation of overrun and the consequent necessity of drawing the sherbet before it is properly frozen.
3. The operation of the scraper at a speed above normal slightly shortens the freezing time and also the time necessary to incorporate 100 per cent overrun and tends to produce a slightly smoother body.
4. Comparing the effect of variations of speed of dasher and scraper in freezing ice cream, the scraper is the more important.

REFERENCES

(1) Dahlberg, A. C. What science shows on whipping mixes. Ice Cream Trade Journal, November, 1928, p. 65.
(2) Lucas P. S. Butterfat, quality, overrun. Ice Cream Trade Journal, July, 1927, p. 51.
(3) Turnbow, G. D. Science shows industry new leads in the processing of ice cream. Ice Cream Trade Journal, August, 1928, p. 50.
(4) Turnbow, G. D. Ice Cream. John Wiley & Sons, p. 185, 186, 187, 188, 189.
(5) Whitaker, R. The influence of the use of butter on the freezing properties of ice cream mix. Journal Dairy Science, January, 1930, p 1.

AN INTERPRETATION OF THE FEEDING STANDARDS FOR GROWING DAIRY CATTLE*

J. B. FITCH

Department of Dairy Husbandry, Kansas Agricultural College, Manhattan

AND

R. H. LUSH

Agricultural Experiment Station, Louisiana State University, Baton Rouge

In experimental work in feeding growing dairy animals over long periods of time, there is a need for definite standards of feed requirements. It is necessary to use some guide; first, to insure that animals receive sufficient amounts of nutrients and, second, in order that trials may be accurately repeated. It is realized that any arbitrary standard is likely to be but an approximation of requirements for growth, especially if little or no attempt is made to consider the source of energy, the quality of the protein, or the mineral and vitamin content of the food. The authors do not propose to criticize existing standards but, rather, to present some experience in interpreting them, believing that other workers may have had the same difficulty. Table 1 shows the requirements as listed in two standard texts.

The Armsby (1) standard expresses in digestible true protein and net energy the requirements for animals of different weights. No figures are listed for animals weighing over 800 pounds. The usefulness of the Armsby standard is limited by the fact that the net energy is not known for all feeds.

The Morrison (2) standard is adaptable to more general use, especially in experimental trials with new feeds or those of varying composition that can readily be analyzed. Also it can be used for larger animals than can the Armsby standard. The Morrison standard is used almost exclusively at this Station for these reasons. However, in figuring the nutrients required for an ani-

---

* Contribution No. 71, Department of Dairy Husbandry, Kansas State Agricultural College.

Received for publication July 20, 1930.

mal of a given weight, inconsistencies are encountered because of the abrupt change in requirements from one 100 pound class to the next. The requirements for both protein and total digestible nutrients are expressed in maximum and minimum pounds required for 100 pounds live weight. The range in protein is given in order that the feeder may take advantage of fluctuations in price of protein feeds. However, to get the average requirement for experimental work, one would ordinarily average the two extremes of the class, divide by 1000 and multiply by the actual weight of the animal. This is accurate for an animal weighing near the middle of the weight class. But using the same method, it will be found that an animal weighing near the upper limit of one class will be shown to require more protein than another animal weighing near the lower limit of the next higher class. That is to say the feed requirement so calculated will be least for the heavier animal. This variation is illustrated by the dotted line in figure 1, in which the maximum, minimum, average, and calculated amounts of protein required for animals weighing from 150 to 950 pounds is plotted. The average which is based on the maximum and minimum for each 50 pound class accurately represents the protein requirements for any size animal. Where

TABLE 1

Nutrient requirements for growing dairy animals

WEIGHT	MORRISON, PER DAY PER 1000 POUNDS		ARMSBY, PER DAY		
	Digestible crude protein	Total digestible nutrients	Weight	Digestible true protein	Net energy
pounds	*pounds*	*pounds*	*pounds*	*pounds*	*therms*
100-200	2.9-3.2	17.0-19.0	100	0.40	3.1
200-300	2.6-2.9	16.5-18.5	135	0.45	3.4
300-400	2.3-2.6	15.5-17.5	165	0.55	3.6
400-500	2.0-2.3	14.5-16.5	275	0.70	4.1
500-600	1.8-2.0	13.8-15.8	325	0.75	4.4
600-700	1.7-1.9	13.0-15.0	400	0.80	5.1
700-800	1.6-1.8	12.2-14.2	550	0.85	6.4
800-900	1.5-1.7	11.4-13.4	700	0.85	7.6
900-1,000	1.3-1.5	10.6-12.6	800	0.85	8.2

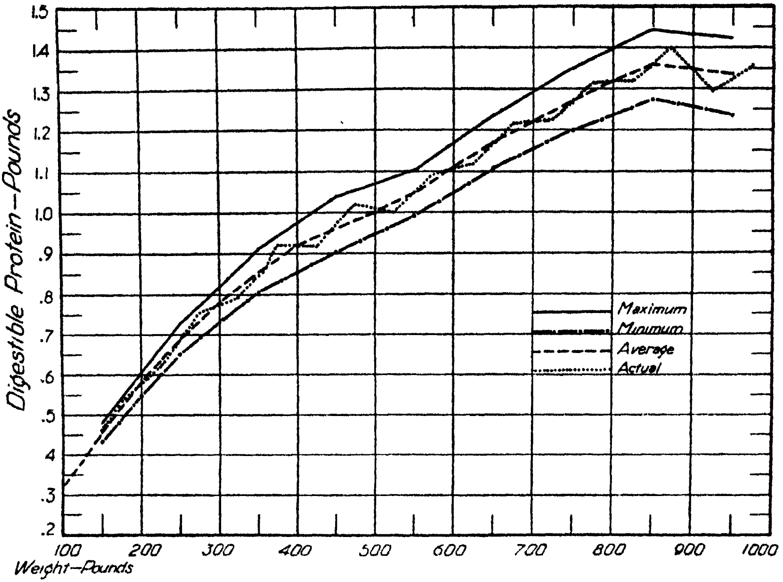


Fig. 1. Digestible Protein Requirements

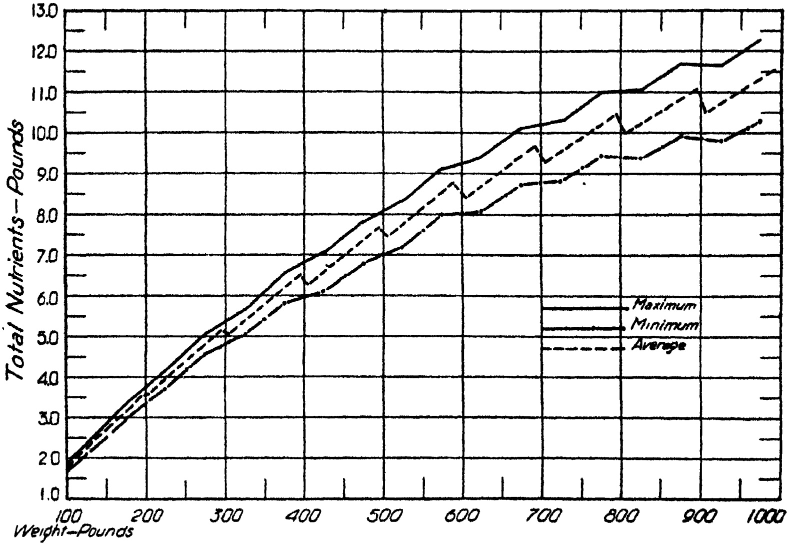


Fig. 2. Total Digestible Nutrient Requirements

this curve crosses a base line of a given weight, the digestible protein required will be found expressed in the legend on the left. This curve is satisfactory until 900 pounds is reached. For animals reaching maturity at about 950 pounds as with the Jersey breed this figure may be correct as the protein requirement depends on rate of growth and maintenance. But from Armsby (3) and from Morrison's standard for growing fattening steers it is reasonable to believe that the total amount of protein used increases slightly with any considerable increase in body weight. On this assumption we have completed the curve of requirements to 1200 pounds using the rate of regression from 100 to 800 pounds. An animal of each 50 pound class requires a smaller percentage of protein than the preceding class until the increase amounts

TABLE 2

Morrison Standard for Growth (with suggested changes)

WEIGHT	DIGESTIBLE CRUDE PROTEIN	TOTAL DIGESTIBLE NUTRIENTS
pounds	*pounds*	*pounds*
100	0 320	1 9
150	0 457	2 70
200	0 580	3 55
250	0 687	4 375
300	0 780	5 10
350	0 857	5 775
400	0 920	6 40
450	0 967	6 975
500	1 00	7 575
550	1 045	8 14
600	1 11	8 64
650	1 17	9 10
700	1 225	9 52
750	1 275	9 90
800	1 320	10 24
850	1 335	10.54
900	1 350	10 80
950	1 365	11 02
1,000	1 37	11 20
1,050	1 38	11 34
1,100	1 39	11 44
1,150	1 41	11 50
1,200	1 42	11 53

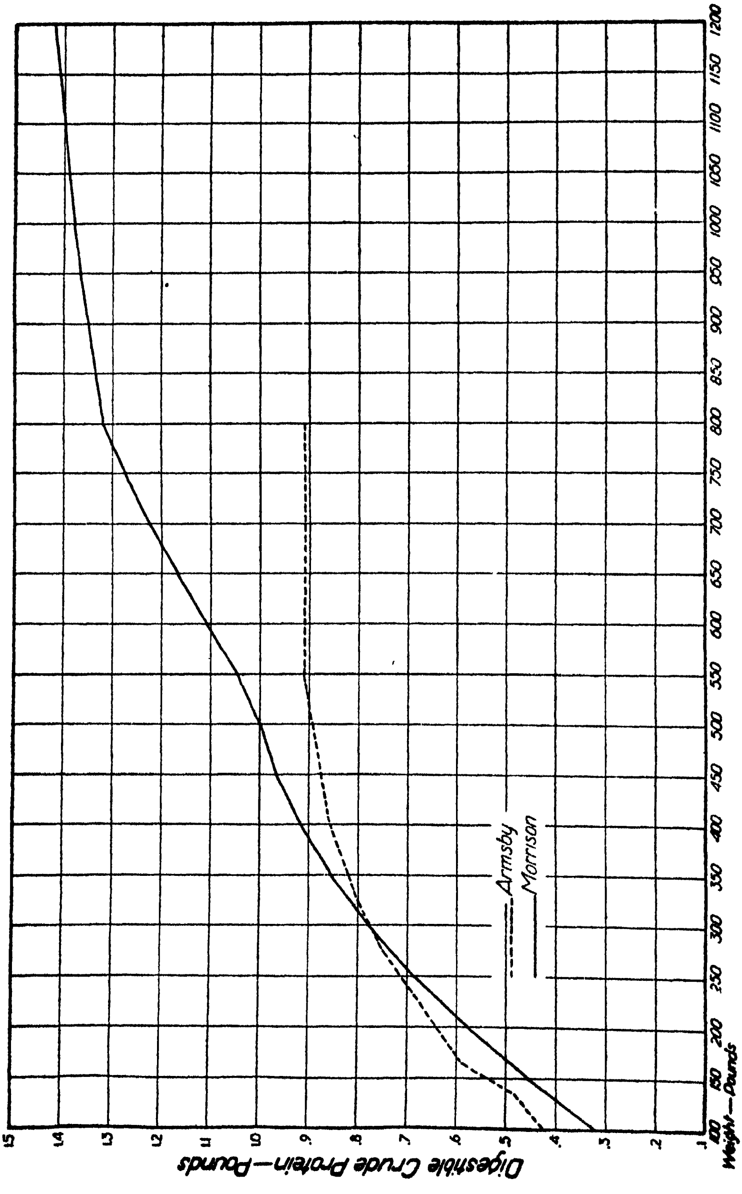


FIG. 3. COMPARISON OF ARMSBY AND MORRISON PROTEIN REQUIREMENTS FOR GROWTH

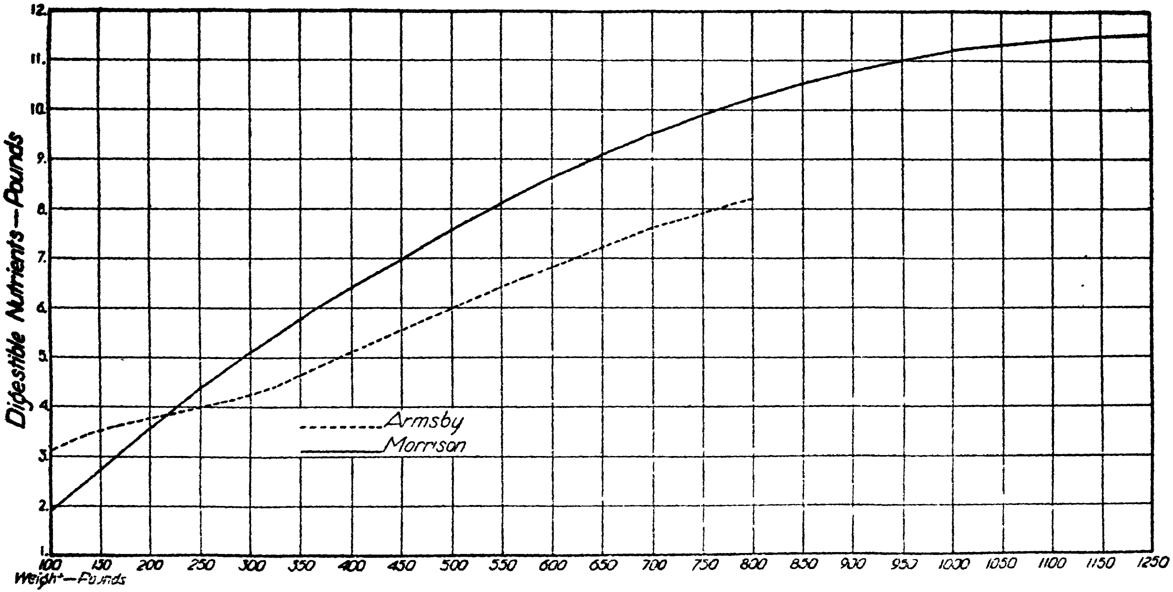


FIG. 4. COMPARISON OF ARMSBY AND MORRISON TOTAL NUTRIENT REQUIREMENT FOR GROWTH

to only 0.7 per cent at 1200 pounds. These percentage increases are slightly smaller than for growing fattening steers of the same weight. This curve, then, as shown in figure 3 is probably conservative in estimating the protein required for large dairy animals. According to the Missouri Station (4), Holstein heifers required an average of only 69.9 per cent of the Wolff-Lehnmann (Morrison) requirements for protein while Jerseys required 101.6 per cent. Granted that there is a breed difference in metabolism due to a difference in the ratio of body surface to weight and other possible factors, the 1000 pound Holstein heifer should have more total protein than the 900 pound Holstein. Similar reasoning should hold for other differences in weight between animals of the same body type or else maintenance requirements are wrong.

Much the same difficulty is encountered in figuring the amount of total digestible nutrients required. If the table is used literally an animal weighing just over 600 or 700 pounds, for example, would require less nutrients than one weighing slightly less than these amounts. This is shown by the dotted line in figure 2, which shows the total digestible nutrient requirements for animals weighing between 100 and 975 pounds. The maximum and minimum requirements were figured for animals of each 25 pound class. Certainly an animal's requirements do not vary as markedly as shown here nor did Morrison make that assumption. But the only fair way to interpret the figures for ready use is by taking averages for 50 and 100 pound classes and plotting a curve as shown in figure 4. The nutrients required may then be read directly from the intersection of the curve and base or weight line. Here again it is necessary to extend the curve to 1200 pounds. As the increase in nutrients of each 50 pound class from 200 to 950 pounds was 0.04 of a pound less than the increase over the previous class, this rate was used from 950 to 1200 pounds. The rate of increase in nutrients for growing fattening steers is considerably higher than this, indicating that the curve is very conservative in estimating nutrients necessary for growing large dairy animals. Table 2 presents the original data from which the solid line of figures 3 and 4 are plotted.

We do not have sufficient data to say whether the Morrison standard would supply enough nutrients for normal or average growth. Normal growth itself is affected by many local factors. This standard is generally believed to be liberal in its allowance of nutrients, however, and work of the Missouri Station indicates this to be true. In figures 3 and 4 is presented a comparison of the Armsby and Morrison standards for growth, in terms of the latter. Corn was selected as a basis for converting digestible true protein to terms of digestible crude protein. For example, in the case of a 410 pound animal, 0.8 pounds of digestible true protein is required according to Armsby. Dividing 0.8 by 0.07, the amount of digestible true protein in one pound of corn, the result indicates that 11.42 pounds of corn would be necessary to supply this protein. Then by multiplying by 0.075 the equivalent amount of digestible crude protein is found to be 0.857 pounds. This figure, then, represents the Armsby requirement expressed in the Morrison term. Similarly, all the stated requirements were converted and listed in figure 3.

The curve given then represents the Armsby figures for protein on a concentrate basis with no attempt to correct or extend it. The same process was used in converting net energy to equivalent figures of total digestible nutrients in figure 4. Here the difference is not so marked. But with alfalfa hay as a basis for comparison, the Armsby standard is close to or exceeds that of Morrison. That is, an 800 pound dairy heifer fed on alfalfa hay alone according to net energy requirements will get more total digestible nutrients and protein than called for in the Morrison standard. The choice of a standard or guide to follow will depend somewhat on the type of experimental feed used.

SUMMARY

Any feeding standard should be looked upon only as a guide. The Morrison standard for growing dairy animals, however, can be readily used in the majority of experimental trials and it is hoped more quickly interpreted by the use of table 2 plotted on a large scale as shown in figures 3 and 4.

REFERENCES

(1) Armsby, H. P.: The Nutrition of Farm Animals, p. 713. The Macmillan Co., New York, 1917.

(2) Henry and Morrison: Feeds and Feeding, 18th Edition, p. 747. The Henry-Morrison Company, Madison, Wisconsin.

(3) Armsby, H. P.: Nutrition of Farm Animals, p. 378–379. The Macmillan Co., New York, 1917.

(4) Report of the University of Missouri Agricultural Experiment Station, 1922, Bul. 197, pp. 57-58.

THE EFFECT OF FEEDING MENHADEN (FISH) OIL ON THE SECRETION OF MILK AND THE COMPOSITION OF BUTTER FAT IN THE DAIRY COW[*]

J. B. BROWN AND T. S. SUTTON

Laboratory of Physiological Chemistry and the Department of Animal Husbandry, Ohio State University, Columbus

The problem of the effect of diet on the quality and quantity of milk from the dairy cow has been one of great interest and the subject of numerous investigations, including those of Wing (1), Anderson (2), Eckles and Palmer (3), Mattick (4), Petersen (5), Maynard and MaCay (6) and Petersen, Palmer and Eckles (7). Milk as a glandular secretion and as a food is unique not only because it contains at least two biologically complete proteins, casein and lactalbumin, and a characteristic sugar, lactose, but also because it contains a fat that is composed of glycerides of oleic acid and of the entire series of saturated fatty acids up to stearic. Few of these compounds are ordinarily found in the food of the cow. Casein, lactalbumin, lactose and the lower fatty acids are apparently, therefore, products of the synthetic action of the mammary gland. The higher fatty acids, palmitic, stearic and oleic, appear to be derived for the most part from the blood lipids and hence from the fat of the diet.

In a continuance of a series of investigations on the fate of highly unsaturated fatty acids in animal metabolism (8), the primary object of the present investigation was to trace definitely the passage of the characteristic highly unsaturated fatty acids of menhaden (fish) oil from the food into butter fat in the dairy cow. These acids consist of a mixture of the following (9):

Hexadecatrienoic	$C_{16}H_{26}O_2$
Clupanodonic	$C_{18}H_{28}O_2$
Arachidonic	$C_{20}H_{32}O_2$
Eicosapentenoic	$C_{20}H_{30}O_2$
Docosapentenoic	$C_{22}H_{34}O_2$
Docosahexenoic	$C_{22}H_{32}O_2$

---

[*] Received for publication July 30,1930.

They may be easily identified by bromination as acids or methyl esters in cold ether, when white, insoluble polybromides are formed which may be analysed for bromine, and which, in the case of the methyl esters of the lower members of the series up to arachidonic, give characteristic melting points. These acids, further, are non-toxic and are deposited readily in the body fat of the rat (8) and the hog (10) when they are included in the diet. One of them, arachidonic, occurs normally in rat fat (11), human fat (12), and lard (13) in very small quantities. Comparatively large amounts of it occur in the lipids of the brain and some of the glandular tissues.

A number of investigators have demonstrated the passage of abnormal fatty acids into butter fat when these acids are included in the diet. Caspari (14) found iodized fat in butter when the iodized fat was fed. Engel (15) claims to have found sesame oil in human milk after the oral ingestion of this oil. Bowes detected arachidic acid ($C_{20}H_{40}O_2$) in goat butter twelve hours after peanut oil was eaten (16). Gogitidse (17) found that sheep after eating linseed oil gave milk fat containing as much as 33 per cent of the oil, and that this oil readily passed into human milk. Hempseed fat greatly depressed lactation. Channon, Drummond and Golding (18) have recently studied the effect of cod liver oil on lactation and quality of butter. In general their findings are confirmed by the work reported below. It should be noted that chemically the cod liver oil used by these investigators is very similar to menhaden oil.

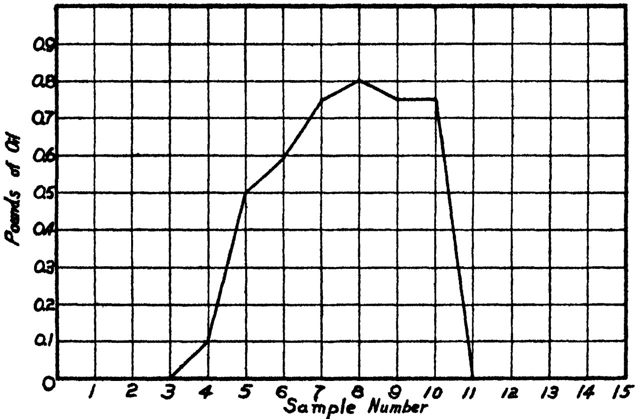
In the present investigation a dairy cow after a six-day control period was fed on a diet containing considerable quantities of menhaden oil for fourteen days. After this period of experimental feeding the control diet was resumed for ten days. The general results of the fish oil feeding were to greatly reduce the milk production, the percentage of butter fat in the milk and total butter fat yield. Upon discontinuing the oil, partial recovery in milk yield occurred with complete recovery in the percentage of butter fat. As a result of chemical analysis of the butter fat samples, it was found that the normal butter of this cow yielded small amounts of ether insoluble bromine addition products, a result which was confirmed by the analysis of several specimens of commercial butter. A small amount of a highly unsaturated fatty acid occurs, therefore, in normal butter. This acid may be arachidonic, or a mixture of arachidonic with other acids and has not been described previously. During the period of fish oil feeding the quantity of polybromides obtained increased about six times, showing that small amounts of the fish oil acids passed into the butter.

EXPERIMENTAL PART

Details of feeding

The feeding experiment was not completely satisfactory, mainly because of difficulty in getting the cow to eat the menhaden oil diet. Since, however, the main object of the work as stated above was attained, the results will be reported.

A purebred Holstein, weighing 1340 pounds in her sixth month of lactation, was used in the experiment. For the preceding month she had averaged 18.5 pounds of milk per day, testing 3 per cent butter fat. For the first six days of the experiment she was fed 6 pounds of basal mixture (Basal ration: 9 parts dried distillers grain, 5 hominy feed, 2 oats, 2 bran), 3⅜ pounds of starch per day, and in addition 6 pounds beet pulp, ½ pound molasses and no. 1 green alfalfa hay ad libitum. Milk samples were collected for two day periods, tested for butter fat, and separated. After six days of control ration (3 samples) 1½ pounds of the menhaden oil (a commercial specimen of dark amber color of not unpleasant taste, except for its decided fishy odor) was substituted for the starch, the rations being calorifically isodynamic. Difficulty was experienced in getting the cow to take the feed. The oil in the ration was then lowered until the cow found the mixture palatable, with an attempt to keep the ration of equal calorific value by increasing the allowance of molasses and beet pulp which also aided in making the food succulent. The animal was kept on the fish oil diet for fourteen days. Toward the end of the feeding period she became such a slow eater that corn silage was used in replacing part of the beet pulp, thus slightly increasing the protein content of the ration. Since the oil consumption was necessarily variable the average daily consumption is shown in figure 1. It should be noted in this chart, as in other charts and tables given below, that the sample numbers represent two-day specimens. After fourteen days of oil feeding, the oil was withdrawn from the ration and starch again added. The effect was followed for an additional ten days.



Average Amounts of Menhaden Oil Consumed Daily

FIG. 1

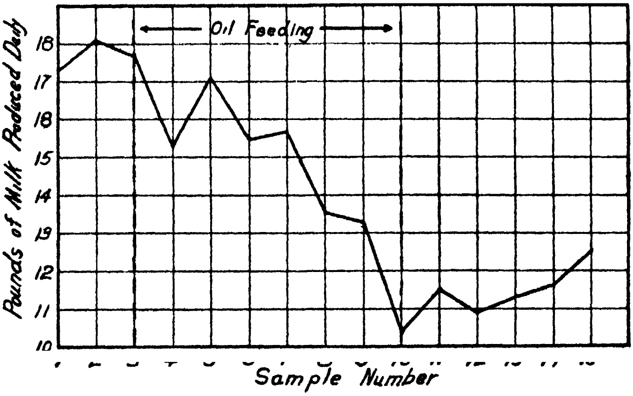
Methods of Analysis

Butter fat was estimated by the Babcock method. The cream from two-day specimens of milk was worked up for the butter fat, which was dried by warming under reduced pressure and filtered through cotton to remove any particles of curd. Iodine, saponification, and Reichert-Meissl numbers were determined on the fat by the usual methods. The remainder of the butter fat after analysis was converted into methyl esters by boiling eighteen hours with an excess of dry methyl alcohol containing HCl gas in solution. The esters were distilled under reduced pressure (10 mm.) and analysed. The remainder of the esters was brominated in cold ether, the ether-insoluble bromides removed by centrifugation, and thoroughly washed in the centrifuge bottle with fresh cold ether. They were weighed and the polybromide number (per cent yield of polybromides) was calculated. Bromine determinations were made by the Parr peroxide bomb method. Such small amounts of bromides were obtained from the control samples 1, 2, and 3 that these were combined for bromine analysis.

DISCUSSION OF RESULTS

Effect of milk production (fig. 2)

The daily milk production during the six-day control period was at the 17–18–pound level. This decreased rapidly to 10.4 pounds on the fish oil diet. Upon discontinuing the oil, recovery was slow, the output being only 12.5 pounds ten days after. Two weeks after the end of the experiment on the regular herd ration the yield was still only 12.1 pounds. A permanent drying off effect resulted under this experimental regime.

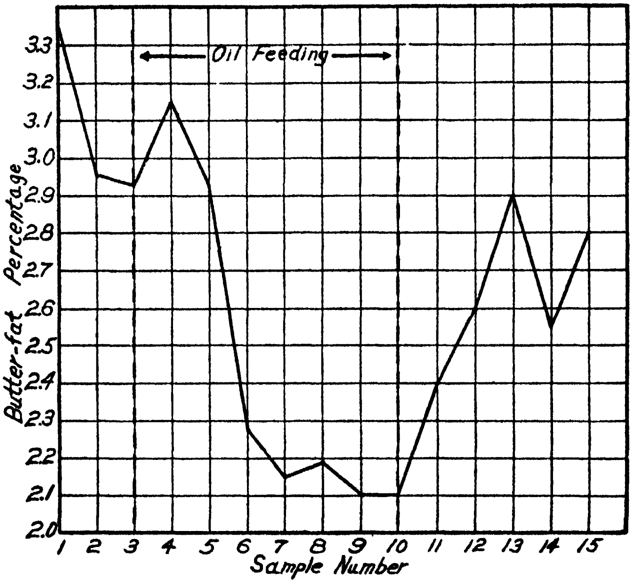


Effect of Oil Feeding on Milk Production

FIG. 2

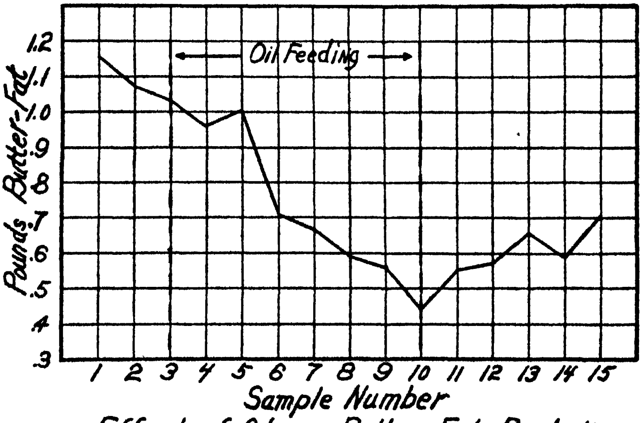
Effect on percentage of butter fat and total butter fat (figs. 3 and 4)

During the fish oil feeding period the butter fat dropped from 3.0 to 2.1 per cent, with almost complete recovery during resumption of the control ration. Two weeks later butter fat was normal.



Effect of Oil Feeding on Percentage Butter-Fat in Milk

FIG. 3



Effect of Oil on Butter-Fat Production

FIG. 4

The effect of the oil on total butter fat production was even more marked with a drop from 1.1 pound of fat per day to 0.45 pound. The butter fat yield did not recover completely after fish oil was discontinued because of the drying-off effect mentioned previously.

The data available are not sufficient to explain this very remarkable effect. Three causes might be given for the effect of the oil on the quality and quantity of the milk. (*a*) A relatively high fat content of the diet. This question has been discussed many times by previous investigators with no final conclusions. (*b*) The high vitamin content of the oil, especially vitamin D. While menhaden oil contains only about one-half the vitamin D of cod liver oil, the amount of this vitamin ingested was quite considerable and might account for the change. This specimen of oil was not tested for vitamin D. (*c*) The effect of the highly unsaturated acids. However, no deleterious physiological action has been observed previously on feeding menhaden oil. Rats have been fed on diets containing up to 30 per cent of this oil with no apparent harm. Our data offer no means of deciding these questions although we feel that D hyper-vitaminosis is the most likely cause. They are important not only to the dairyman but also to those interested in the nutrition of nursing women. The prescribing of cod liver oil and of viosterol (irradiated ergosterol, vitamin D) is a common practice with the medical profession. The effect of large doses of viosterol on the secretion of human milk may be very important and even harmful.

Chemical changes in the butter fat (table 1)

Chemically the butter fat during the control period was not unusual, except for the slightly higher than average iodine number. The results in table 1 are in agreement with the hypothesis that the fatty acid radicals of the food pass into the butter, appearing therein as glycerides. The body processes which account for the synthesis of the lower saturated fatty acids are probably not greatly modified. Instead, more molecules of higher fatty acids are present in the blood and in increased amounts pass into the butter. The result, therefore, of feeding an oil with a high content of unsaturated fish oil fatty acids with high iodine number and molecular weight is to raise the iodine number of the butter as much as 20 points (50 per cent increase), to lower the saponification number, hence raise the molecular weight of the acids and to greatly lower the content of volatile soluble acids by increasing the content of the higher acids. The analytical data on sample (7), for example, were so changed from the normal that analytically the fat was not butter fat. This specimen actually remained liquid in the laboratory for several days. A very marked but not complete return of analytical data to normal occurred after the oil feeding was stopped.

TABLE 1

Analyses of butter

SAMPLE	IODINE NUMBER	SAPONIFICATION NUMBER	REICHERT-M NUMBER
1	41.40	224.68	25.9
2	41.89	224.22	25.0
3	42.42	224.69	26.7
Fish oil begins			
4	45.89	222.35	23.1
5	48.52	222.63	23.4
6	62.78	218.02	20.5
7	59.71	219.18	17.7
8	62.41	218.35	17.6
9	60.45	219.65	19.6
10	57.65	217.36	19.0
Fish oil ends			
11	53.49	214.60	17.0
12	47.78	215.68	17.1
13	43.76	217.30	17.9
14	41.87	217.93	19.0
15	40.67	220.92	20.3
Menhaden oil	176.61	187.5	1.64

The methyl ester analyses (table 2) are significant mainly with respect to polybromide number and percentage of bromine in the bromide determinations. We were surprised to find that the three control samples gave appreciable yields of ether-insoluble bromides (an average polybromide number of 0.427). Calculated as arachidonic acid by the method described previously (19) this would amount to about 0.54 per cent of this acid in the esters. The bromine analysis, 67.12 per cent, was, however, 0.34 per cent too high for methyl octobromoarachidate (66.78), while the average for samples 4, 5, 8, and 9 was 68.14, an increase of 1.02 per cent due to the menhaden oil. The maximum polybromide number was given by sample 9, i.e., 2.59, an increase of about six times due to the fish oil. After ten days of control diet the polybromide number returned nearly to its original value although the bromine analysis was still high.

TABLE 2

Analyses of the methyl esters of butter

SAMPLE	IODINE NUMBER	SAPONIFICATION NUMBER	POLYBROMIDE NUMBER	PER CENT BROMINE
1	39.25	207.47	0.385	
2	40.32	204.31	0.442	
3	38.36	205.83	0.453	67.12
Fish oil feeding begins				
4	42.18	205.23	0.651	66.98
5	50.39	206.72	0.586	67.44
6	62.72	205.61	1.765	64.91
8	62.17	207.20	2.590	69.33
9	58.62	207.32	2.047	68.81
10	56.08	205.18	1.416	
Fish oil feeding ends				
12	46.08	204.16	0.645	67.62
14	41.87	205.33	0.595	68.57
Menhaden oil	172.60	187.02	53.5	69.50

The appearance of ether-insoluble bromides in samples 1, 2, and 3 indicated the presence in butter of an acid similar to arachidonic. This conclusion was further verified in three specimens of commercial butter. A 5 pound specimen (Fairmont Creamery), for example, gave methyl esters (iodine number 33.45, saponification number, 208.9) which yielded a polybromide number of 0.24, with 66.97 per cent bromine. These esters contained, therefore, about 0.30 per cent of acid calculated as arachidonic. The question of the identity of this acid was made doubtful, however, by its melting point behavior. Pure methyl octobromoarachidate melts rather sharply at 228° to 230° (uncorrected). The bromides obtained from the butter were compared with a specimen of the pure bromide, and were found to *only partially* melt at 234° to 235° with gas formation which along with the slightly high bromine analysis suggests the presence of more highly unsaturated acids. We have demonstrated, therefore, the presence in normal butter of a fatty acid similar to arachidonic, possibly a mixture of this acid with others more unsaturated. The complete identification of this acid or mixture would require working up of 50 to 100 pounds of butter, and was not further studied. It should be stated here that several other specimens of butter were tested for arachidonic acid with results that were similar but usually slightly higher.

SUMMARY

1. The feeding of menhaden oil to a dairy cow lowered milk production, the percentage of butter fat, and total butter fat. After resumption of control diet recovery to normal took place slowly. There was a tendency toward drying off the animal.
2. The characteristic highly unsaturated acids of the menhaden oil passed into the butter fat in small amounts.
3. The analytical constants of the butter changed to those of a mixture of butter with menhaden oil.
4. Several specimens of normal butter have been shown to contain small quantities (0.3 per cent) of a highly unsaturated fatty acid similar to arachidonic acid.

REFERENCES

(1) Wing, H. H.: On the effect of feeding fat to cows. Bulletin 92, Cornell Univ. Agr. Expt. Sta., 1895.
(2) Anderson, Leroy: The relation of food to milk fat. Ibid., Bulletin 173 (1899).

(3) Eckles, C. H., and Palmer, L. S.: Influence of the plane of nutrition of the cow upon the composition and properties of milk and butterfat. Missouri Agr. Expt. Sta. Research Bull. 25.

(4) Mattick, Elfrida C. V.: The chemical composition of milk of cows receiving cod-liver oil. Biochem. Jour., xxii, 144 (1928).

(5) Petersen, W. E.: The immediate influence of feeds upon the quantity and quality of cow's milk. I. The effect of ground flax. This journal, x, 70 (1927).

(6) Maynard, L. A., and McCay, C. M.: The influence of a low fat diet upon fat metabolism during lactation. Jour. Nutrition, ii, 67 (1929).

(7) Petersen, W. E., Palmer, L. S., and Eckles, C. H : The synthesis and secretion of milk fat. I. The time of milk and fat secretion. II. An analytical study of the fat of the bovine mammary gland. III. A study of the activity of the perfused surviving gland, with special reference to fat. Amer. Jour. Physiol., xc, 573, 582, 592 (1929).

(8) Brown, J. B , and Rawlins, A L.: The effects of feeding whale oil on the depot fat of the white rat. Proc. Soc. Exp Biol. Med., xxvi, 704 (1929); Brown, J. B., and Morris, S. G.: The effect of varying amounts of menhaden oil in the diet on the composition of the body fat of the white rat. The storage of highly unsaturated fatty acids. Jour. Nutrition, ii, 509 (1930).

(9) Brown, J. B., and Beal, G. D.: The highly unsaturated fatty acids of fish oils. Jour. Amer. Chem. Soc., lxv, 1289 (1923).

(10) Brown, J. B.: Jour. Biol Chem , xc, 133 (1931)

(11) Eckstein, H. C.: The influence of diet on the body fat of the white rat. Jour Biol. Chem , lxxxi, 613 (1929).

(12) Eckstein, H. C.: The fatty acids in the subcutaneous fat of man Ibid , lxiv, 797 (1925).

(13) Brown, J. B , and Deck, E. M.: The occurrence of arachidonic acid in lard. Jour. Amer Chem. Soc., lii, 1135 (1930).

(14) Caspari, W.: Engelmann's Arch. Suppl., Ein Beitrag zur Frage nach der Quelle des Milchfettes, 267 (Quoted from Leathes—The Fats (1925)

(15) St. Engel: Arch. f. Kinderheilkunde. Nahrungsfett und Milchfett, xlii, 194 (1906).

(16) Bowes, O. C.: The rate of passage of fatty acid of foods into the mammary gland of the goat Jour. Biol. Chem., xxii, 11 (1915).

(17) Gogitidse, S.: Z. f. Biol., xlv, 365 (1904); xlvi 403 (1905).

(18) Channon, H. J., Drummond, J. C., and Golding: The effects of giving certain oils in the daily diet of cows on the composition of butter fat. Jour. Analyst., xlix, 311 (1924).

(19) Brown, J. B.: Arachidonic acid in the lipids of thyroid, suprarenal and spleen. Jour. Biol. Chem., lxxxiii, 777 (1929).

THE EFFECT OF DILUTION ON THE TITRATABLE ACIDITY OF COWS' MILK*

H. H. SOMMER AND JULES MÉNOS†

Department of Dairy Husbandry, University of Wisconsin

In determining the titratable acidity of milk various procedures are used. Some titrate the milk directly; others dilute the measured sample with definite amounts of water ranging from 1 to 10 volumes; still others dilute the measured sample with indefinite amounts of water used for rinsing the pipette. If such dilution affects the result as various investigators have reported, then this factor in the "acid test" should not be ignored as is now very commonly the case.

The writers' attention was directed to this subject by several instances. In one case, discrepancies in the titratable acidities reported on the same samples of milk by two different laboratories were traced to the fact that one of the laboratories titrated the milk without dilution, while the other diluted the measured samples with two volumes of water. In another case, several large milk plants frequently referred to their milk as having acidities of 0.10 to 0.12 per cent; and comparing the acidities with those mentioned in neighboring plants (0.15 to 0.17 per cent), the operators had come to the false belief that their milk supply was superior. It was found that they determined the titratable acidity by titrating 9 cc. of milk plus 90 cc. of distilled water. These instances admirably illustrate the unfortunate consequences of ignoring dilution as a factor in the "acid test."

As a consequence of the above observations, the present work was undertaken. The results in the literature seem quite conclusive, but further emphasis of this subject appeared desirable.

---

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station. Received for publication July 31, 1930.

† This article is based on the experimental work of the Master of Science thesis, University of Wisconsin, June, 1930, by the junior author.

TABLE 1

The effect of dilution on the titratable acidity as shown by various investigators

INVESTIGATORS	YEAR	SAMPLE NUMBER	UNDILUTED ACIDITY	DILUTED ACIDITY	DIFFERENCE
(Dilution with 1 volume of water)					
			per cent	*per cent*	*per cent*
Berberich	1907	1	0.1305	0.1192	0.0113
		2	0.1462	0.1205	0.0257
		3	0.1305	0.1125	0.0180
Söldner	1888	1	0.1350	0.1225	0.0125
Siegfeld	1904	1	0.2070	0.1800	0.0270
		2	0.1467	0.1287	0.0180
Hanne	1904	1	0.1233	0.1044	0.0189
		2	0.1467	0.1287	0.0180
(Dilution with 2 volumes of water)					
Berberich	1907	1	0.1305	0.1102	0.0203
		2	0.1462	0.1237	0.0225
		3	0.1305	0.1080	0.0225
		4	0.1520	0.1282	0.0238
Soldner	1888	1	0.1350	0.0990	0.0360
Siegfeld	1904	1	0.2070	0.1630	0.0440
		2	0.1638	0.1260	0.0378
Hanne	1904	1	0.1233	0.0972	0.0261
		2	0.1467	0.1206	0.0261
		3	0.1548	0.1278	0.0270
		4	0.1611	0.1287	0.0324
		5	0.5778	0.5742	0.0026
(Dilution with 3 volumes of water)					
Berberich	1907	1	0.1305	0.0990	0.0315
		2	0.1462	0.1170	0.0292
		3	0.1305	0.1125	0.0180
(Dilution with 4 volumes of water)					
Berberich	1907	1	0.1305	0.0945	0.0360
		2	0.1452	0.1125	0.0327
		3	0.1305	0.0945	0.0360
		4	0.1520	0.1125	0.0395

TABLE 1—*Concluded*

INVESTIGATORS	YEAR	SAMPLE NUMBER	UNDILUTED ACIDITY	DILUTED ACIDITY	DIFFERENCE
(Dilution with 5 volumes of water)					
			per cent	*per cent*	*per cent*
Söldner	1888	1	0.1350	0.0832	0.0518
(Dilution with 10 volumes of water)					
Rice and Markley	1927	3	0.216	0.149	0.067
		1	0.185	0.122	0.063
		26	0.180	0.104	0.076
		2	0.176	0.117	0.059
		4	0.168	0.113	0.055
		15	0.095	0.054	0.041
		18	0.086	0.050	0.036

A further aim of the present study was to offer a more detailed explanation of this dilution effect.

REVIEW OF LITERATURE

That the addition of water to the measured sample of milk lowers the titratable acidity has been shown by Söldner (1), Plaut (2), Hanne (3), Siegfeld (4), Berberich (5), Rice and Markley (6) and others. The results reported by these investigators have been compiled in table 1, and show that dilution lowers the titratable acidity and that this effect becomes progressively more pronounced as the dilution is increased.

It is of interest to note that Berberich studied the effect of dilution on both milk, and milk plus neutral potassium oxalate. In the former, dilution lowered the titratable acidity, but in the latter, dilution was practically without effect.

Several explanations have been offered for the effect of dilution Söldner attributed it to the hydrolysis of the calcium caseinate. Rice and Markley concluded that it is due to the hydrolysis of both the caseinates and phosphates, Barthel (8) explains it through the use of an equilibrium equation:

$$3\,Ca^{++} + 2HPO_4^{--} \rightleftharpoons Ca_3(PO_4)_2 + 2\,H^+$$

stating that dilution shifts the equilibrium in such a manner that the titratable acidity and hydrogen ion concentration are decreased. One of us (9) attributed the effect of dilution to a decrease in the amount of tri-calcium phosphate precipitated during the titration. Thus we may conclude that while there is good agreement among investigators as to the effect of dilution, there is no general accepted explanation of this effect.

EXPERIMENTAL

The magnitude of the dilution effect

In order to demonstrate the effect of dilution, the titratable acidities of a number of fresh milk samples were determined and compared with the acidities found in the same samples after dilution with 1 volume and 9 volumes of distilled water.

The milk samples used were obtained from the University herd; they were taken from individual cows of four different breeds in order to obtain relatively wide differences in composition and in titratable acidity. Precautions were taken to prevent any developed acidity.

In the case of each sample, the following titrations were made with N/10 sodium hydroxide: (1) 10 cc. milk, (2) 10 cc. milk plus 10 cc. distilled water, and (3) 10 cc. milk plus 90 cc. distilled water. A one per cent alcoholic solution of phenolphthalein was used as the indicator; 3 drops were used for the 10 cc. milk, and where water was added the amount of indicator was increased in proportion. The titrations were carried to the first definite, and relatively permanent shade of pink. The cubic centimeters of N/10 sodium hydroxide used were converted into percentage of lactic acid, assuming for convenience that the 10 cc. pipette delivered 10 grams of milk.

The results obtained with 19 different samples of milk are given in table 2. The three titrations in the order given above showed averages of 0.1721, 0.1493, and 0.1098 per cent lactic acid, respectively. These results represent a decrease in titratable acidity of 0.0228 per cent when the milk was diluted with an equal volume of water, and a decrease of 0.0623 per cent when diluted with 9 volumes of water. Fourteen other samples were titrated undiluted and diluted with an equal volume of water with essentially the same results; the average acidity of the undiluted samples was 0.1831 per cent, as compared with an average of 0.1643 per cent when the same samples were diluted with an equal volume of water. As an average of the 33 samples, dilution with an equal volume of water decreased the titratable acidity 0.0211 per cent. The results obtained are in good agreement with those found in the literature as given in table 1.

TABLE 2

The effect of dilution on the titratable acidity of milk

NUMBER OF COW	A 10 CC. MILK		B 10 CC MILK + 10 CC WATER		C 10 CC. MILK + 90 CC. WATER		DIFFERENCES DUE TO DILUTION	
	N/10 NaOH	Acidity	N/10 NaOH	Acidity	N/10 NaOH	Acidity	A-B	A-C
	cc.	*per cent*	*cc.*	*per cent*	*cc.*	*per cent*	*per cent*	*per cent*
99	1.3	0.117	1.10	0.099	0.70	0.063	0.018	0.054
23x	1.4	0.126	1.10	0.099	0.75	0.067	0.027	0.059
39	1.5	0.145	1.25	0.112	0.95	0.085	0.033	0.060
81	1.5	0.145	1.30	0.117	0.95	0.085	0.028	0.060
6	1.5	0.145	1.35	0.121	1.00	0.090	0.024	0.065
73	1.85	0.166	1.60	0.144	1.20	0.108	0.022	0.058
3y	1.9	0.171	1.70	0.153	1.20	0.108	0.018	0.063
2y	1.9	0.171	1.70	0.153	1.30	0.117	0.018	0.054
1	1.9	0.171	1.70	0.153	1.30	0.117	0.018	0.054
12	1.9	0.171	1.75	0.157	1.30	0.117	0.014	0.054
2x	1.9	0.171	1.65	0.148	1.20	0.108	0.023	0.063
11	1.9	0.171	1.70	0.153	1.25	0.112	0.018	0.059
25	2.0	0.180	1.80	0.162	1.20	0.108	0.018	0.072
12	2.0	0.180	1.75	0.157	1.30	0.117	0.023	0.063
21	2.2	0.198	2.00	0.180	1.40	0.126	0.018	0.072
27	2.2	0.198	1.90	0.171	1.40	0.126	0.027	0.072
14	2.3	0.207	2.00	0.180	1.50	0.135	0.027	0.072
35	2.4	0.212	2.00	0.180	1.60	0.144	0.032	0.068
20	2.5	0.225	2.20	0.198	1.70	0.153	0.027	0.072
Average.........		0.1721		0.1493		0.1098	0.0228	0.0623

Calcium phosphate and the dilution effect

In view of Berberich's finding that dilution of oxalated milk did not affect the titratable acidity, and in view of the explanations for the dilution effect by Söldner, Rice and Markley, Barthel, and one of us, it was decided to study the behavior of solutions containing calcium and phosphates, to determine their behavior during titration, with and without dilution.

As a preliminary experiment, four solutions were prepared and titrated, undiluted and diluted with 9 volumes of water. The solutions were: (1) 2.537 grams mono-calcium phosphate and 1.866 grams calcium chloride per liter, (2) 2.537 grams mono-calcium phosphate per liter, (3) 1.866 grams calcium chloride per liter, and (4) a lactic acid solution, approximately N/5. The results are given in table 3. Solution 1 contained 0.15 per cent CaO and 0.15 per cent P_2O_5 and is therefore fairly representative of the calcium and phosphate content of milk. Solutions 2 and 3 respectively represent the amounts of mono-calcium phosphate and calcium chloride required to make Solution 1, but dissolved separately. Solution 4, lactic acid, was included as a matter of interest.

TABLE 3

The effect of dilution on titratable acidity of various solutions

SOLUTION		TITRATABLE ACIDITY		
Number	Composition, grams per liter	A 25 cc undiluted	B Diluted, 25 cc + 225 cc H_2O	Difference, A-B
		cc N/10 NaOH	*cc N/10 NaOH*	
1	2.537 grams $Ca(H_2PO_4)_2$ + 1.866 grams $CaCl_2$	8.65	7.45	−1.20
2	2.537 grams $Ca(H_2PO_4)_2$	6.30	5.90	−0.40
3	1.866 grams $CaCl_2$	0.10	0.95	+0.85
4	Approximately N/5 lactic acid	46.20	47.10	+0.90

In the case of Solutions 3 and 4, dilution increased the titratable acidity; this result can readily be explained on the basis of hydrolysis. Solution 2, mono-calcium phosphate, showed a decrease in acidity on dilution; this might conceivably also be explained on the basis of hydrolysis, but it should be recorded that precipitation was observed during the titration, and the amount of precipitate was apparently less in the diluted sample.

A significant indication is gained from a comparison of Solutions 1, 2, and 3, titrated undiluted. Solution 2 had a titer of 6.3 cc., and Solution 3 had a titer of 0.1 cc.; yet when the two were combined as in Solution 1, a titer of 8.65 cc. N/10 NaOH was obtained. Similarly these solutions, titrated after dilution with 9 volumes of water, give a significant indication. Solution 3, calcium chloride, had an increased titer on dilution, yet when this amount of calcium chloride was added to Solution 2 to produce Solution 1, a greater decrease in titer on dilution resulted (compare the decrease in titer due to dilution in Solutions 1 and 2). As will be discussed more fully later, these results can be explained only on the basis of tri-calcium phosphate precipitation.

In order to demonstrate the behavior of solutions containing calcium and phosphates more clearly, electrometric titrations of Solution 1 were undertaken. In the preliminary electrometric titrations it was found that precipitation of tri-calcium phosphate occurred, that the precipitation was quite slow and that there is a decrease in the pH of the solution as precipitation progresses. Wendt and Clarke (10), and Holt, LaMer, and Chown (11) have reported a similar observation; in electrometric titration of similar solutions these investigators found it necessary, after each addition of alkali, to wait several days before making the pH reading, if consistent results were to be obtained.

As a result of the above, the following procedure was adopted: A series of samples was prepared by placing 25 cc. of Solution 1 into each of 25 screw cap jars, and adding accurately measured amounts of N/10 NaOH in such a manner as to produce a series of samples with successively larger additions of NaOH from 0 to 10 cc. A similar series of samples was prepared but containing 25 cc. of Solution 1 and 225 cc. of distilled water. The jars, securely closed to prevent evaporation, were allowed tc stand about 12 hours; from each jar a portion of the clear solution was then pipetted off, taking care not to include any precipitate, and used for the pH determinations. The precipitate, in those cases where any had formed, was collected by filtering through a weighed Gooch crucible, using the clear filtrate to complete the transfer from the jar. The crucibles were then dried to constant weight. The results are given in figure 1, showing both the electrometric titration curves and the amounts of precipitate.

It will be noted in figure 1 that there is a break in each of the titration curves, a decrease in pH in spite of an increase in the amount of sodium hydroxide added, and that this break coincides with the occurrence of the precipitate. Further, the break occurs much earlier in the undiluted mono-calcium phosphate-calcium

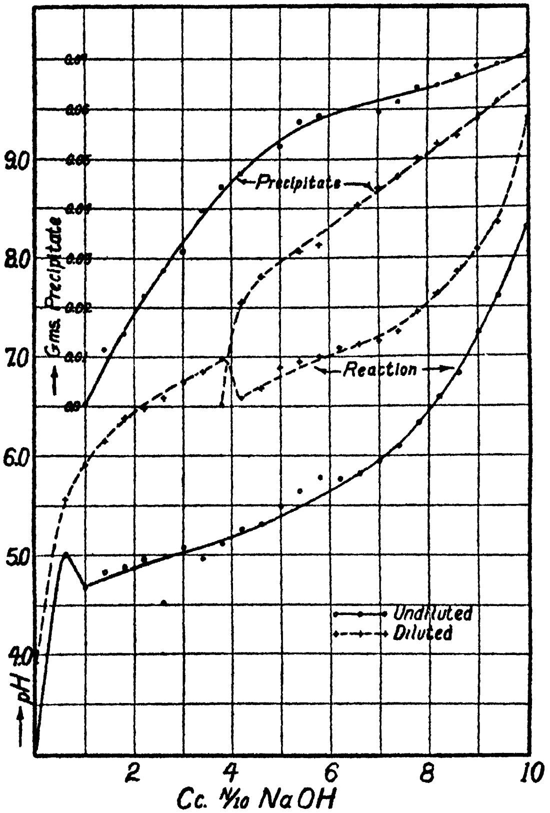


Fig. 1. Titration and Precipitation Curves of a Mono-calcium Phosphate-calcium Chloride Solution Titrated Undiluted and Diluted with Nine Volumes of Water

chloride solution than in the solution diluted with 9 volumes of water. It is clear that the precipitation of tri-calcium phosphate plays a significant rôle in the titration of such solutions.

Although not strictly related to the present subject, it is of interest to note here that when freshly prepared portions of Solutions 1 and 2 were heated at 62.8°C. for 30 minutes, Solution 2 showed only a slight precipitate while Solution 1 showed an appreciable precipitate. The reaction of Solution 2 changed from pH 6.64 to 5.18, and Solution 1 from pH 6.72 to 4.85. These results suggest an explanation for the results observed by Van Slyke and Baker (12) that the reaction of milk remains unchanged by pasteurization in spite of the escape of carbon dioxide, while in carbon dioxide free milk, pasteurization causes a decrease in pH. A precipitation of tri-calcium phosphate would cause a decrease in pH but the loss of carbon dioxide from the milk has the opposite effect; when carbon dioxide free milk is pasteurized, the effect of tri-calcium phosphate precipitation then becomes evident.

The phenolphthalein end-point and the dilution effect

On determining the reaction of undiluted and diluted milk samples after they had been titrated to the phenolphthalein end-point in the usual manner, it was found that the pH in the case of diluted samples was lower. As a result, a number of milk samples were titrated to the phenolphthalein end-point as already described, but using 25 cc. of milk for the undiluted samples and 25 cc. milk plus 225 cc. distilled water for the diluted samples. After the samples had been carefully titrated to the same shade of pink as judged by the eye, the reaction was determined electrometrically, using the hydrogen electrode. The results are given in table 4, and show that the end-point is reached at an appreciably lower pH in the diluted samples.

The reason for the above differences in reaction at the end-point in undiluted and diluted milk was sought in the effect of salts and proteins on the phenolphthalein; dilution would reduce the "salt and protein errors." The reaction at the phenolphthalein end-point was determined in four samples of milk, titrated, diluted with 9 volumes of water, and diluted with 9 volumes of a 0.7 per

TABLE 4

The reaction at the phenolphthalein endpoint in undiluted and diluted milk samples

UNDILUTED SAMPLES		DILUTED SAMPLES, 9 VOLUMES OF WATER ADDED		DIFFERENCE, pH
Cc. N/10 NaOH	Phenolphthalein endpoint, pH	Cc. N/10 NaOH	Phenolphthalein endpoint, pH	
5.2	8.59	4.2	7.72	0.87
5.2	8.48	4.4	7.97	0.51
5.3	8.61	4.3	7.94	0.67
5.5	8.46	4.9	8.30	0.16
5.6	8.63	5.0	8.24	0.39
5.25	8.34	3.9	8.22	0.12
5.6	8.39	3.9	8.16	0.23
5.85	8.73	4.7	8.15	0.58
6.05	8.49	4.0	8.01	0.48
6.0	8.39	4.0	8.16	0.23
6.2	8.42	4.8	8.07	0.35
6.45	8.67	4.5	7.97	0.70

TABLE 5

Effect of casein on the phenolphthalein endpoint

COMPARISON NUMBER	UNDILUTED CASEIN SOLUTIONS, PHENOLPHTHALEIN ENDPOINT, PH	CASEIN SOLUTIONS + 9 VOLUMES OF WATER, PHENOLPHTHALEIN ENDPOINT, pH	DIFFERENCE
1	8.30	8.10	0.20
2	8.49	8.32	0.17
3	8.51	8.25	0.26
4	8.62	8.34	0.28
5	8.49	8.30	0.19
6	8.62	8.34	0.28
7	8.73	8.19	0.54
8	8.63	8.04	0.59
9	8.57	8.15	0.42
10	8.47	8.25	0.22
11	8.52	8.24	0.28
12	8.50	8.32	0.18
13	8.69	8.35	0.34

cent common salt solution. The samples diluted with water had reactions at the phenolphthalein end-point as follows: pH 8.01, 8.16, 8.16, and 8.22. The same samples diluted with 0.7 per cent common salt solution had reactions as follows: pH 7.92, 7.94, 7.93, and 8.06. Thus at the higher salt concentration, the end-point is reached at a slightly lower pH, than diluted milk. The effect of casein concentration on the end-point was observed by titrating casein solutions, undiluted and diluted with 9 volumes of water, and determining the reaction at the end-point. The casein solution used, was made by dissolving 4 grams of purified casein in 100 cc. lime water, dialyzing 24 hours, and neutralizing to pH 6.60 with N/10 HCl–M/20 sodium citrate solution. The undiluted and diluted casein solutions were titrated at the same time in each comparison in order to match the end-points as closely as possible. Thirteen such comparisons were made, and in each case the end-point in the diluted casein solution was reached at a lower pH. The results are given in table 5. The effect of casein concentration on the end-point of the indicator accounts for the greater part of the difference in the pH at the end-point in undiluted and diluted milk.

The above experiments should be regarded as preliminary; more experiments on the effect of other milk salts and proteins on the phenolphthalein end-point, are desirable, but time did not permit their undertaking.

DISCUSSION

The results on the effect of dilution on the titratable acidity of milk, as found in this work, (table 2) are in good agreement with those reported in the literature (table 1). Dilution of the measured sample of milk with an equal volume of distilled water was found to lower the titratable acidity 0.211 per cent; dilution with 9 volumes of water lowered it 0.0623 per cent.

An important factor in this effect of dilution is the decreased precipitation of tri-calcium phosphate during the titration. A solution representative of milk with respect to calcium and phosphate content (Solution 1, table 3) also showed a decreased titer on dilution with 9 volumes of water; the decrease in titer was equivalent to 0.043 per cent lactic acid. That this decreased titer is due to less tri-calcium phosphate precipitation is indicated by the results given in figure 1.

The manner in which the precipitation of tri-calcium phosphate affects the titratable acidity is indicated more clearly by figure 2, reproduced from the work of Holt, LaMer and Chown. Figure 2 gives the titration curves of phosphoric acid, titrated with sodium hydroxide and calcium hydroxide. It will be noted that in the titration with sodium hydroxide, the first equivalent of phosphoric acid has been neutralized at about pH 4.5, and the second at pH 8.8. This well known titration curve leads to the common statement that in the titration of phosphoric acid or acid phosphates, we have titrated only to the di-basic salt at the phenolphthalein end-point. However, in the titration with calcium hydroxide, it will be seen from figure 2 that all three of the acid hydrogens of the phosphoric acid have been titrated long before the pH of the phenolphthalein end-point has been reached. This can only occur only because of the precipitation of tri-calcium phosphate.

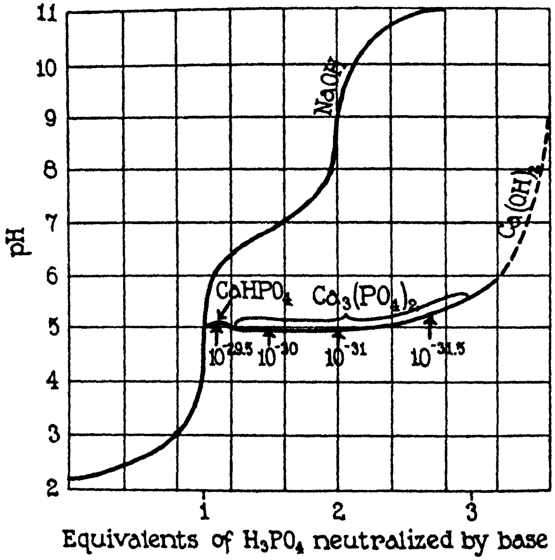


FIG. 2. TITRATION CURVES OF PHOSPHORIC ACID TITRATED WITH SODIUM HYDROXIDE AND CALCIUM HYDROXIDE

(Reproduced from Holt, LaMer and Chown)

The precipitation of tri-calcium phosphate can occur only if there are tertiary phosphate ions present. It is a common error to say that below pH 4.5 there are no secondary phosphate ions, and below pH 8.8 there are no tertiary phosphate ions present in the solution (see fig. 2). The presence of tertiary phosphate ions below pH 8.8 must necessarily obtain as shown by calculations from the dissociation constants of phosphoric acid. It is true that the tertiary phosphate ion concentration may be insignificant and can be ignored for such purposes as calculating buffer mixtures, but when the progressive removal of tertiary phosphate ions by precipitation is involved, even a very small concentration of such ions becomes of utmost significance.

TABLE 6

Calculated tertiary phosphate ion and calcium ion concentrations in milk at various reactions

pH	(H^+)	(PO_4^{---})	(Ca^{++})	$(Ca^{++})^3 \times (PO_4^{---})^2$
6.5	3.12×10^{-7}	6.554×10^{-9}	1.07×10^{-2}	5.304×10^{-23}
7.0	1×10^{-7}	3.514×10^{-8}	1.07×10^{-2}	1.515×10^{-21}
7.5	3.12×10^{-8}	1.477×10^{-7}	1.07×10^{-2}	2.694×10^{-20}
8.0	1×10^{-8}	5.058×10^{-7}	1.07×10^{-2}	3.159×10^{-19}
8.5	3.12×10^{-8}	1.673×10^{-6}	1.07×10^{-2}	3.157×10^{-18}

To illustrate the above and to give us a more definite basis for a discussion of the dilution effect, the calculations given in table 6 have been made. In these calculations it has been assumed that the P_2O_5 content of milk is 0.15 per cent and that the phosphates are 70 per cent dissociated, and that the calcium oxide content is 0.15 per cent and that only 40 per cent is diffusible and dissociated. Thus the total phosphate ion concentration is assumed to be 1.50/71 × 0.70 or 0.01477 molar, and the calcium ion concentration is assumed to be 1.50/56 × 0.40 or 0.0107 molar. The tertiary phosphate ion concentration was calculated from the dissociation constants, k_2 and k_3, of phosphoric acid.

The calculations involved are illustrated by the following calculation for pH 6.5 ($H^+ = 3.12 \times 10^{-7}$). From k_2 (1.95×10^{-7})

we know that the ratio of primary to secondary phosphate ion concentrations must be:

$$\frac{(H^+)}{1.95 \times 10^{-7}} \quad \frac{(H_2PO_4^-)}{(HPO_4^{--})} = \frac{3.12 \times 10^{-7}}{1.95 \times 10^{-7}}$$

Knowing the ratio of primary to secondary phosphate ion concentrations, and having assumed that the total phosphate ion concentration is 0.01477, we can now compute the secondary phosphate ion concentration. In this step of the calculation we can ignore the tertiary phosphate ion concentration without introducing a significant error. If the total phosphate ion concentration is 0.01477 and if there are 3.12 parts of primary to 1.95 parts of secondary phosphate, then the secondary phosphate ion concentration will be:

$$\frac{1.95}{3.12 + 1.95} \times 0.01477 = 0.00568$$

From this figure and from k_3 (3.6×10^{-13}) we can now calculate the tertiary phosphate ion concentration at pH 6.5, as follows:

$$\frac{(H^+)}{3.6 \times 10^{-13}} = \frac{(HPO_4^{--})}{(PO_4^{---})}$$

Substituting 3.12×10^{-7} for (H^+), and 0.00568 for (HPO_4^{--}) and calculating for (PO_4^{---}), we get (PO_4^{---}) $= 6.554 \times 10^{-9}$. Similarly the concentrations of tertiary phosphate ions may be calculated for the other reactions shown in the table.

The solubility product of tri-calcium phosphate has been investigated by Holt, LaMer and Chown (11) and by Sendroy and Hastings (13) in connection with blood serum at 38°C. They found that the solubility product is influenced by the presence of other salts; values have been reported ranging from 1×10^{-26} to $1 \times 10^{-32.5}$. We are not justified in applying these values to milk for exact calculations but to demonstrate in a qualitative sense what we may expect in milk is justified. For such a purpose we may take $(Ca^{++})^3 \times (PO_4^{---})^2 = 1 \times 10^{-26}$, as the solubility product of tri-calcium phosphate. Compare the figures in the last column of table 6 with this value; it will be noted that even at pH 6.5, the calculated $(Ca^{++})^3 \times (PO_4^{---})^2$ value exceeds the above solubility product, and that with increases in the pH, the calculated values become still greater. The conclusion is that precipitation of tri-calcium phosphate must be expected during the titration of milk.

The precipitation of tri-calcium phosphate increases the titratable acidity, because, by the removal of tertiary phosphate ions by precipitation, the equilibrium equation,

$$HPO_4^{--} \rightleftharpoons H^+ + PO_4^{---}$$

proceeds to the right; the result is that the third acid hydrogen must be neutralized to the extent that the tertiary phosphate ions are removed by precipitation.

If the milk is diluted to ten times its original volume by the addition of 9 volumes of distilled water, the tertiary phosphate ion and calcium ion concentrations will be lowered to approximately one-tenth of their former value. Precipitation will therefore occur later in the titration and will be considerably less at the phenolphthalein end-point. The result is a lower titratable acidity.

The calculations on which the above conclusion is based, involve assumptions as to the calcium ion concentration, the tertiary phosphate ion concentration, and the solubility product of tri-calcium phosphate. These assumptions might be varied appreciably without impairing the validity of the above conclusion. For example, even if we assume that the calcium ion concentration of milk is only 1 per cent of the total calcium content, or $(Ca^+) = 2.8 \times 10^{-4}$, as Whittier (15) concluded on the basis of calculations, the above calculations will still indicate precipitation of tri-calcium phosphate during the titration to the phenolphthalein end-point. The calculations given above are intended only for the purpose of making the discussion concrete; the object of the discussion is to point out the qualitative facts that precipitation of tri-calcium phosphate is favored as the pH is increased, and that less precipitation of tri-calcium phosphate takes place in more dilute solutions, and to indicate that these facts are involved in the titration of milk.

While this work does not include any experimental results on the effect of casein in the presence of calcium, there are indications that the acidity contributed to the milk by the casein is influenced by the calcium content in a manner similar to the phosphates. Casein acts as a weak polybasic acid, the successive dissociations of which are favored by increases in pH. As a result, casein is capable of combining with additional bases as the neutralization progresses, and thus it contributes to the titratable acidity of milk as Rice and Markley (6) have shown. However, if there is a difference in the solubility of the caseinates of various bases, then the titratable acidity contributed by the casein may be affected by the bases present in a manner similar to the phosphates.

That there is a difference in the solubility of calcium caseinate and sodium caseinate is indicated by the fact that in the presence of both bases, the calcium caseinate is formed. Further evidence is furnished by the work of Osborne (7) who found that calcium caseinate did not filter through a Pukall filter, but on the addition of even small amounts of ammonium or potassium oxalate to such a caseinate solution, the filtrate showed the presence of casein; when sufficient oxalate was added and the solution allowed to stand for several days, the filtrate contained all of the casein.

If we regard the calcium caseinates as insoluble, then we must expect that the caseinates will behave in a manner entirely analogous to the phosphates. Porcher (14) has performed experiments which bear quite directly on this subject; he found as follows:

AMOUNTS OF $CaCl_2$ ADDED	ADDED TO 1 LITER DISTILLED WATER	ADDED TO 1 LITER Ca CASEINATE SOLUTION
grams	*pH*	*pH*
0.0	5.08	6.62
1.0	6.08	6.42
2.0	6.58	6.49

The addition of calcium chloride to water increased the pH, while the addition of the same amount of calcium chloride to calcium caseinate caused a decrease in pH. A possible explanation of this result is that the addition of the calcium ions caused the formation of less soluble calcium caseinates with a resulting further dissociation of the casein in a manner analogous to the behavior of the phosphates.

As has already been pointed out, Berberich's work showed that the dilution effect involves the calcium salts; the titratable acidity of oxalated milk was not affected by dilution. Berberich also found that rather large additions of oxalate are required before the fullest effect of such additions are realized. These facts suggest that in addition to phosphates, there is some other substance, probably casein, involved with the calcium in the titration of oxalated milk and in the dilution effect. If only calcium in relation to phosphates were involved, it would seem that a relatively small addition of oxalate should suffice; because of the rather low solubility product of calcium oxalate and the low concentration of tertiary phosphate ions, large additions of oxalate should not be necessary to prevent the precipitation of tricalcium phosphate. These considerations lend support to the belief that a calcium to casein relationship is involved in the dilution effect. Further experimental evidence on this point would be desirable.

Another factor responsible for the lower titratable acidity of diluted milk samples is the observed fact that the pH at the phenolphthalein end-point is always considerably lower in the diluted samples. Not enough work was done to determine exactly the cause for this observation, but indications are that the main cause is the effect of the proteins on the end-point of the indicator. A high protein concentration shifts the end-point to a higher pH; diluting therefore lowers the pH at the end-point.

Another factor that has been suggested in explanation of the lower titratable acidity of diluted milk samples is the increase in pH when milk is diluted with water. The thought apparently is that since dilution of the milk increases the pH slightly, not as much alkali will be required in the titration because the reaction is slightly closer to the phenolphthalein end-point to begin with. This logic is fallacious as can be demonstrated on the basis of theoretical considerations; it is sufficient here to point out that dilution of an approximately N/5 lactic acid solution (as in table 3) certainly increases the pH, but the titratable acidity is increased.

While the main object of this study was to determine and explain the effect of dilution on the titratable acidity of milk, the facts revealed by the experiments enable us to explain other phenomena, viz. (*a*) the fading of the end-point in titrating milk, (*b*) the titratable acidity of condensed milk, and (*c*) the neutralization with lime.

It is a common experience in titrating milk to observe that the pink color of the phenolphthalein end-point fades quite rapidly. This is commonly explained by saying that carbon dioxide is absorbed from the air, but the inadequacy of this explanation is indicated by the fact that other solutions, which are quite as good solvents for carbon dioxide, are not subject to such rapid fading. A more logical explanation for the rapid fading is based on tri-calcium phosphate precipitation. Results given here and in the literature show that milk or a phosphate solution will become more acid (lower pH) as a result of phosphate precipitation. The results of Wendt and Clarke (10), verified in the present study, show that several hours are required for this precipitation to reach equilibrium. Hence the explanation for the fading is as follows: When the milk has been brought to the pink color, the precipitation of the tri-calcium phosphate has not reached equilibrium, further precipitation occurs within a comparatively short time and the resulting drop in pH explains the fading.

The titratable acidity of concentrated milk is usually higher than calculated from the ratio of concentration and the acidity of the original milk. This is true in spite of the fact that carbon dioxide is known to have been expelled in the condensing process. This observation is to be explained by the same factors involved in the dilution effect, but in this case we are dealing with an increased concentration and these factors therefore cause an increased titratable acidity.

In the neutralization of milk or cream with lime of definitely known alkalinity, it is common to note that the acidity attained is not as low as calculated. This is due to the fact that the addition of lime increased the calcium ion concentration with the result that in subsequent titrations there is more tri-calcium phosphate precipitated and hence a higher titratable acidity is obtained than was calculated from the original titration and the known amount of lime added. It is for a similar reason that titrating milk with standard barium hydroxide solution leads to higher titratable acidities than titration with sodium hydroxide, as shown by Siegfeld (4), and Plaut (2).

SUMMARY AND CONCLUSIONS

1. Dilution of the measured sample of milk lowers the titratable acidity; dilution with an equal volume of water caused an average decrease of 0.0211 per cent, and dilution with 9 volumes of water, an average decrease of 0.0623 per cent.
2. It has been demonstrated that in solutions containing calcium and phosphates in amounts representative of milk, precipitation of tri-calcium phosphate will occur during titration with sodium hydroxide, and that such precipitation increases the titer. By calculation it has been shown that a similar precipitation must be expected in the titration of milk.
3. The greater part of the dilution effect is attributed to a decrease in the amount of tri-calcium phosphate precipitated during the titration.
4. There are indications that a relationship of calcium to casein, similar to that of calcium to phosphates, is involved in the dilution effect.
5. A lower pH was observed at the phenolphthalein end-point in the titration of diluted milk. This is attributed to a "protein effect" on the indicator.
6. The rapid fading of the end-point in titrating milk is attributed to the precipitation of tri-calcium phosphate.
7. The fact that the titratable acidity of concentrated milk is higher than calculated from the acidity of the original milk and the ratio of concentration, in spite of the loss of carbon dioxide, is attributed to the reverse of the same factors involved in the dilution effect.
8. The neutralization of milk or cream with lime of known alkalinity does not lower the acidity to the calculated point because the increased calcium ion concentration causes greater precipitation of tri-calcium phosphate in subsequent titration.

REFERENCES

(1) Söldner, Friedrich 1888 Die Salze der Milch und ihre Beziehungen zu dem Verhalten des Kaseins. Laudwirtschaftlichen Versuchs-Stationen, xxxv, 351–436.

(2) Plaut, H. C. 1891 Ueber die Beurtheilung der Milch nach dem Verfahren der Sauretitrirung. Archiv für Hygiene, xiii, 133–172.

(3) Hanne, R. 1904 Die Aciditat der Kuhmilch. Milch-Zeitung, xxxiii, 659–660, 725–727.

(4) Siegfeld, M. 1900 Ueber die Acidatatsbestimmung in der Milch. Molkerei-Zeitung (Hildesheim), xiv, 205–207.

(5) Berberich, F. M. 1907 Beitrage zur Titration von Milchflussigkeiten. Molkerei-Zeitung (Hildesheim), xxi, 1359–1362.

(6) Rice, Frank E., and Markley, Alton L. 1924 Natural acidity in milk. Jour. Dairy Sci , vii, 469–483.

(7) Osborne, W. A. 1901 Caseinogen and its salts. Jour. Physiol., xxvii, 398.

(8) Barthel, Ch. 1920 Die Methoden zur Untersuchung von Milch und Molkerei Produkten. Paul Parey, Berlin, p. 98.

(9) Sommer, H. H. 1929 The milk salts and their importance in dairy work; the acidity of milk. The Milk Dealer.

(10) Wendt, Gerald L., and Clarke, Alfred H. 1923 An electrometric study of the neutralization of phosphoric acid by calcium hydroxide. Jour. Amer. Chem. Soc., xlv, 881–887.

(11) Holt, L. Emmett, Jr., LaMer, Victor K., and Chown, H. Bruce 1925 Studies in calcification. I. The solubility product of secondary and tertiary calcium phosphate under various conditions. Jour. Biol. Chem , lxiv, 509–565.

(12) Van Slyke, Lucius L , and Baker, John C 1919 Carbonic acid and carbonates in cows' milk. Jour Biol. Chem., xl, 335–344.

(13) Sendroy, Julius, Jr., and Hastings, A. Baird 1927 Studies of the solubility of calcium salts. Jour. Biol. Chem., lxxi, 783–846.

(14) Porcher, Ch. 1930 Le Lait au point de vue colloidal. Le Lait, 2 Quai Chauveau, Lyon (5^e), p. 211

(15) Whittier, E. O. 1929 The solubility of calcium phosphate in fresh milk. Jour. Dairy Sci., xii, 405–409.

AGGLUTININ, COMPLEMENT, AND CHEMICAL COMPOSITION OF THE COLOSTRUM OF THE COW*

M. SATO, K. OGURA AND H. IKEJIMA

Zootechnical Institute, Hokkaido Imperial University, Sapporo, Japan

INTRODUCTION

That the colostrum contains a large number of antibodies which are transmitted to new-born cattle and thus play a very important part in assuring the healthful growth of the latter is a well known fact, Bertarelli (1), Famulener (2), Zubrzycki und Wolfsgruber (3), Reymann (4) Little and Orcutt (5). The works of eminent scholars agree in the opinion that antibodies are most abundant in the colostrum obtained immediately after parturition and afterwards they speedily decrease and finally become extinct. What is interesting to state is that although the presence of any antibody in the blood of new-born calves immediately after parturition has never been proved, the colostrum, when first given them, is easily transmitted through the digestive organ without causing any abnormal phenomenon whatever. Consequently it may be asserted that the new-born calves, when fed with the colostrum, will be immune to a variety of organisms. The results of experiments by Smith (8) and others show that sucking calves not fed with the colostrum are easily infected by certain bacteria, particularly *Bacterium coli*, and are apt to die.

Besides antibodies, the presence of complements has been reported in the colostrum (also in the milk of cow suffering from mastitis, Bauer and Sassenhagen), the complement being secreted for a period of over ten days after parturition. The presence of complement is essential for the normal operation of certain antibodies. For instance, bacteriolysin requires the presence of complement for the operation of its bactericidal action. For this reason the presence of complement in the colostrum may be of unusual significance.

---

* Received for publication August 2, 1930.

The milk secreting organ of a milch cow may easily be affected by diverse circumstances producing abnormal conditions. Consequently the percentage of milch cows secreting normal colostra from physical, chemical, and physiological standpoint may be very small. Experiments conducted by one of the authors for upwards of 10 years with colostra obtained from a large number of cows has indicated the prevalence of abnormal colostra, an observation which may explain the variety of previously published investigations concerning colostra. In these cases, all researches and investigations dealing with the antibody content of colostrum will be of little significance unless the so-called normal colostrum is used in the experiments.

The authors have been and are conducting the researches concerning the immunity given by the colostra since January, 1925. The following is a report of the results of chemical analyses, and of the agglutination tests and the investigations as to the presence of complement which they carried out up to March, 1926, with normal colostra obtained from 16 cows of Ayrshire, Guernsey, and Holstein breeds and one sample of milk from a cow infected with mastitis and another after abortion.

PARTICULARS CONCERNING THE COWS

The colostrum used for the experiment was obtained from the cattle of Ayrshire breed kept at the first and from Holsteins and Guernseys kept at the second farms of the Faculty of Agriculture, Hokkaido Imperial University.

The particulars are given in table 1.

PHYSICAL AND CHEMICAL ANALYSIS OF THE COLOSTRA

Throughout the experiments, the color was determined with J. W. Lovibond's tintmeter and the measurement of hydrogen ion concentration by the potentimeter. In the alcohol test, a certain quantity of the colostrum mixed with equal quantity of alcohol (68 per cent) was put into a test tube, the whole contents being slightly shaken. In the test, the degree of coagulation was examined by putting the test tube containing the colostrum into boiling water. The determination of specific gravity exceeding

TABLE 1

Information on the history of the cows used for the experiments

EXPERIMENT NUMBER	BREED	RATE OF BIRTH	MAXIMUM DAILY MILK PRODUCTION FOR EACH LACTATION PERIOD PRIOR TO EXPERIMENTS								
			1	2	3	4	5	6	7	8	9
			pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
1	Ayrshire No. 94	April, 1918	46 5	46 5	43 0						
2	Ayrshire No. 94					30 8					
3	Ayrshire No. 96	June 28, 1921			45 0	42 0	42 0				
4	Ayrshire No. 115	May 28, 1921									
5	Ayrshire No. 110	June 28, 1921	30 5								
6	Ayrshire No. 116	May 1, 1923									
7	Guernsey No. 173	November 30, 1918	25 2	27 7	31 0	34 7	27 9				
8	Guernsey No. 168	September 22, 1916	23 3	18 0	31 4	32 9	40 4	39 7	14 2		
9	Guernsey No. 189	August 9, 1917	18 3								
10	Holstein No. 195	August 9, 1917	37.8	62 9	58 8	59 9	72.9				
11	Holstein No. 237	April 12, 1923									
12	Holstein No. 171	March 10, 1915	39 0	53 5	56 3	60 7	69 5	29 5	80 7		
13	Holstein No. 243	July 28, 1923									
14	Holstein No. 197	November 8, 1917	36 7	50 3	70 4	83 3	77 0				
15	Holstein No. 173	June 28, 1915	35 4	44 5	32 5	35 8	58.5	55 9	48 3		
16	Holstein No. 239	June 23, 1923									
17	Guernsey No. 170	Feb. 15, 1917	24 4	26 5	33 8	46 8	27 8	*			
18	Holstein No. 169	March 2, 1915	42 2	54 0	55 8	59 3	66 1	87 2	37 3	98 0	†

* Inflammation of under following parturition.

† Samples taken after an abortion on the 110th day after conception and 220 days after previous parturition.

1.04 was made by the use of a Westphal balance at the temperature of 15°C., Quevenne-Müller's lactometer being employed in the determination of specific gravity below 1.04, the degrees ascertained being converted to 15°C. In the case of the colostrum secreted immediately after parturition which was too thick and cohesive for the Westphal balance, a pycnometer was used. The determination of total solids, total protein, fat, ash, casein, and a portion of albumin were made according to the ordinary methods. The results of the experiment are shown in table 2.

AGGLUTINATION TEST

Milk serum. Fresh colostrum was coagulated by mixing with such a quantity of rennet solution (rennet solution, pH 5.69, prepared at Hansen's Laboratory, N. Y., U S. A.) that would coagulate the mixture at 40°C. in approximately 40 minutes. After filtration the clear filtrate was used in the tests.

Bacterial sus pension. Two milligrams of a 2 hour growth was mixed with 1 cc. of physiological saline. This suspension was preserved for use as antigen with 1 per cent formalin.

The milk serum was heated for about 30 minutes at 56°C. and after being made inactive the liquid was diluted with physiological salt solution. To this liquid was added an equal quantity of bacterial suspension, the mixture being completely mixed and incubated for 2 hours at 37°C. The liquid was afterward left at room temperature for 22 hours, after which the agglutination-titre was observed. The results of this experiment are shown in table 3.

Demonstration of complement

The experiment was made by means of a horse erythrocyte-rabbit hemolytic system using as complement fresh colostrum from which the greater portion of fat was removed by means of a centrifugal separator, the time required being about 10 minutes and the number of revolutions 3000 per minute.

Suspension of horse erythrocytes. A 1 per cent suspension of washed (three times) horse erythrocytes was used. Amboceptor used in the demonstration process consisted of the blood of rabbits

TABLE 2

The chemical composition of colostra

NUMBER OF EXPERIMENT	NUMBER OF PARTURITION	HOURS AFTER PARTURITION	NUMBER OF MILKING	COLOR			pH	ALCOHOL TEST	BOILING TEST	SPECIFIC GRAVITY	TOTAL SOLID	TOTAL PROTEIN	CASEIN	LACTO-GLOBULIN	ALBUMIN	FAT	LACTOSE	ASH	MILK QUANTITY
				Red	Yellow	Blue													
											per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*
I	4	Immediately after	1	1.5	1.5	0	6.03	+	+	1.071	28.51	18.44	—	—	—	7.37	1.51	1.19	11.0
		4	2	1.4	1.4	0	6.05	+	+	1.057	27.98	16.38	—	—	—	8.48	2.04	1.08	4.0
		12	3	1.3	1.2	0	6.08	+	+	1.053	23.98	11.25	—	—	—	9.51	2.15	1.07	3.5
		24	4	1.0	0.8	0	6.08	+	—	1.042	14.60	8.17	—	—	—	2.30	3.17	0.96	14.0
		29	5	1.1	1.0	0	5.92	+	″	1.027	24.85	8.08	—	—	—	13.12	2.80	0.85	4.5
		34	6	1.0	0.7	0	5.91	+	″	1.036	16.66	6.50	—	—	—	5.86	3.43	0.87	6.5
		48	7	0.6	0.5	0.7	5.74	+	″	1.038	14.81	6.59	—	—	—	3.84	3.56	0.82	16.5
		53	8	0.6	0.5	0.7	6.13	+	″	1.036	19.23	7.29	—	—	—	7.72	3.33	0.89	6.0
		58	9	0.6	0.5	0.7	6.14	+	″	1.036	14.66	5.91	—	—	—	4.44	3.54	0.77	4.0
		72	10	0.6	0.5	0.7	6.03	+	″	1.032	14.25	6.52	—	—	—	3.41	3.61	0.71	17.5
		77	11	0.6	0.5	0.7	6.11	—	″	1.029	19.93	5.91	—	—	—	9.98	3.27	0.77	5.8
II	5	19 hours before		1.1	1.0	0	6.09	+	+	1.076	25.96	19.36	13.98		5.38	3.52	2.11	0.97	12.0
		Immediately after	1	1.0	1.2	0	6.04	+	+	1.068	30.00	17.29	8.41		8.88	9.05	2.45	1.21	2.8
		9	2	1.0	0.9	0	—	+	+	1.046	16.02	4.99	4.22		0.77	6.98	3.19	0.86	2.6
		33	3	—	—	—	6.24	+	—	1.037	13.94	4.67	4.10		0.57	6.01	2.48	0.78	11.8
		48	4	—	—	—	6.35	+	″	1.0375	14.43	3.54	3.09		0.45	5.97	3.83	0.79	6.0
		51	5	—	—	—	—	+	″	1.0343	14.84	4.23	4.01		0.32	5.52	4.23	0.76	4.5
		57	6	—	—	—	6.25	+	″	1.0337	—	—	—		—	—	—	—	16.0
		62	7	—	—	—	6.40	+	″	1.037	—	—	—		—	—	—	—	7.0

II	5	76	8	—	—	—	—	+	"	1 0334	—	—	—	—	—	—	—	4.5
		81	9	—	—	—	6 19	+	"	1 034	—	—	—	—	—	—	—	18 5
		105	10	—	—	—	6 23	+	"	1 038	—	—	—	—	—	—	—	7.5
		110	11	—	—	—	—	—	"	—	—	—	—	—	—	—	—	4.9
III	6	18	3	0 4	0 4	0	6 38	+	–	1 051	18.48	10 14	4 04	6.10	5 47	1.81	1.06	3.5
		33	4	0 4	0 3	0 4	6 26	+	"	1 041	—	—	—	—	—	—	—	5.8
		37	5	0 4	0 4	0 4	6 11	+	"	1 0365	15 02	5 65	3.97	1 68	4 34	4.13	0 90	4 0
		57	6	0 1	0 1	0 4	6 24	+	"	1 034	—	—	—	—	—	—	—	13.7
		61	7	0 1	0 1	0 4	6 45	+	"	1 032	15 88	4 98	4 47	0.51	6 38	3.66	0.86	6 5
		66	8	0 1	0 1	0 5	—	+	"	1 034	—	—	—	—	—	—	—	5.0
		81	9	0 2	0 1	0 5	—	–	"	1 036	—	—	—	—	—	—	—	16 2
		85	10	0 2	0 2	0 4	—	"	"	1 032	14 58	4 46	3 68	0 78	5 27	4 11	0 74	7.1
		90	11	0 1	0 1	0 4	—	"	"	1 033	—	—	—	—	—	—	—	5.8
		105	12	—	—	—	—	"	"	1.032	—	—	—	—	—	—	—	20 3
		109	13	—	—	—	—	"	"	1 034	—	—	—	—	—	—	—	7.6
IV	1	12	3	0 4	0 4	0	6 45	+	–	1 046	22 78	9 96	7 22	3 74	—	—	1.04	1 6
		39	4	0 4	0 2	0 4	6 52	+	"	1 039	16 04	4 19	3 29	0 90	—	—	0 90	2.8
		43	5	0 5	0 4	0 4	6 46	+	"	1 035	17.06	3 67	3 05	0.62	—	—	0.81	1 0
		63	6	0 3	0 1	0 3	6 51	+	"	1 034	—	—	—	—	—	—	—	6 8
		67	7	0 4	0 1	0 3	6 51	+	"	1 033	—	—	—	—	—	—	—	2 8
		72	8	0 4	0 1	0 3	6 52	+	"	1 033	—	—	—	—	—	—	—	3 0
		76	9	0 2	0 2	0 3	—	+	"	1 036	11.70	3 83	3 15	0 68	—	—	0 77	8.4
		96	10	0 3	0 1	0 4	—	+	"	1 034	—	—	—	—	—	—	—	3 2
		100	11	0 3	0 1	0 5	—	+	"	1 0335	—	—	—	—	—	—	—	3 5
		115	12	0 4	0 2	0 5	—	–	"	1 033	—	—	—	—	—	—	—	12.0
		120	13	0 3	0 1	0 7	—	"	"	1 033	13 72	3 92	3 43	0 49	—	—	0 88	4 7
		130	14	0 3	0 2	0 9	—	"	"	1 035	—	—	—	—	—	—	—	3 6

TABLE 2—*Continued*

NUMBER OF EXPERIMENT	NUMBER OF PARTURITION	HOURS AFTER PARTURITION	NUMBER OF MILKING	COLOR			pH	ALCOHOL TEST	BOILING TEST	SPECIFIC GRAVITY	TOTAL SOLID	TOTAL PROTEIN	CASEIN	LACTO-GLOBULIN	ALBUMIN	FAT	LACTOSE	ASH	MILK QUANTITY
				Red	Yellow	Blue													
											per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	pounds
V	2	Immediately after	1	1 1	1 5	0	—	+	+	1 061	—	—	—		—	—	—	—	11.0
		4	1	0 6	0 6	0 4	—	+	+	1 0525	—	—	—		—	—	—	—	7.3
		10	3	—	—	—	—	+	—	—	—	—	—		—	—	—	—	4.7
		24	4	0 3	0 3	0 7	—	+	"	1 0396	—	—	—		—	—	—	—	14 7
		28	5	0 7	0 2	0 5	—	+	"	1 0356	—	—	—		—	—	—	—	6 7
		34	6	—	—	—	—	+	"	1 036	—	—	—		—	—	—	—	5.2
		48	7	—	—	—	—	+	"	1 0355	—	—	—		—	—	—	—	18 8
		52	8	—	—	—	—	+	"	1 0364	—	—	—		—	—	—	—	7.1
		58	9	—	—	—	—	—	"	1 0347	—	—	—		—	—	—	—	5 8
		72	10	—	—	—	—	"	"	1 0334	—	—	—		—	—	—	—	21 0
		76	11	—	—	—	6 18	"	"	1 0347	—	—	—		—	—	—	—	7 6
		82	12	—	—	—	—	"	"	1 0324	—	—	—		—	—	—	—	5 5
VI	1	Immediately after	1	0 8	1 0	0	—	+	+	1 071	27 47	18 66	6 69	5 50	6 47	5.97	1 83	1 01	0 2
		17	2	0 6	0 8	0	7 43	+	—	1 045	11 83	4 50	3 16	0 30	1 04	3 87	2.64	0 82	1.1
		21	3	0 3	0 3	0 6	6 26	+	"	1.0395	12 49	4 26	3 68	0 08	0 50	3 60	2.84	0 79	5.3
		34	4	0 4	0 3	0.1	6 14	+	"	1 038	12 06	—	4.16	—	0 51	3 61	3 05	0 73	5.5
		39	5	0 7	0 4	0 9	6 38	+	"	1 036	12 43	3 17	2 62	0 03	0 32	4.31	4 17	0.78	3.3
		44	6	0 3	0 1	0 3	6 09	+	"	1 038	12 72	—	3 04	—	0 57	5 04	3 32	0.75	8.4
		57	7	0 1	0 1	0 6	7 89	+	"	1 037	—	—	—	—	—	—	—	—	4.0
		62	8	0 3	0 1	0 4	7 94	+	"	1 025	—	—	—	—	—	—	—	—	2.0

VI	1	67	9	0 1	0	1 0	8 01	+	″	1 035	—	—	—	—	—	—	—	—	9 8
		80	10	—	—	—	8 06	+	″	1 037	—	—	—	—	—	—	—	—	5 7
		85	11	0 1	0 2	2 0	8 04	—	″	1 035	—	—	—	—	—	—	—	—	3 5
		90	12	0 1	0 2	2 0	7 90	″	″	1 032	—	—	—	—	—	—	—	—	10 0
		103	13	0 2	—	0 9	8 02	″	″	1 035	—	—	—	—	—	—	—	—	5 2
		108	14	0 1	0 1	0 9	8 09	″	″	1 030	—	—	—	—	—	—	—	—	4.3
VII	5	Immediately after	1	2 0	2 0	0	6 17	+	+	1 0692	22 40	15 23	—	—	—	1 04	2 00	1 13	4 5
		24	6	1 8	1 8	0	6 15	+	+	1 0630	22 75	12 83	—	—	—	6 88	2 35	0 69	1 5
		28	10	1 4	1 4	0	6 17	+	—	1 0424	16 20	6 99	—	—	—	5 04	3 35	0 82	2 6
		72	14	1 1	1 1	0	6 20	—	″	1 0310	19 28	7 27	—	—	—	8 07	3 09	0 85	3 7
		96	18	0 8	0 8	0	6 22	″	″	1 035	15 50	5 79	—	—	—	5 76	3 14	0 81	3 7
		120	22	0 8	0 6	0 6	6 38	″	″	1 0347	14 59	6 62	—	—	—	3 78	3 48	0 71	4 0
		144	26	0 8	0 5	0 5	6 38	″	″	1 0322	18 36	6 94	—	—	—	7 38	3 24	0 80	4 8
VIII	8	Immediately after	1	—	—	—	—	+	+	1 071	25 23	16 81	5 17		11 64	6 29	1 05	1 08	13 5
		25	6	—	—	—	—	+	—	1 039	14 54	4 83	3 84		0 99	7 56	1 27	0.87	6 0
		49	10	—	—	—	—	—	″	1 035	13 47	3 43	2 54		0 89	6 06	3 09	0.89	2 7
		73	14	—	—	—	—	″	″	1 034	13.59	4 03	3 35		0 68	4 46	4 20	0.90	7.8
		97	18	—	—	—	—	″	″	—	—	—	—		—	—	—	—	6.5
		121	22	—	—	—	—	″	″	—	—	—	—		—	—	—	—	7 6
IX	2	Immediately after	1	—	—	—	—	—	—	1 0562	27 40	19 11	—		—	3 88	—	0 97	12 2
		15	3	—	—	—	—	″	″	1 036	16 14	4 87	3.14		1 73	4 79	5 75	0 72	3 0
		39	7	—	—	—	—	″	″	1 028	15 06	3 75	2 14		0 61	4 63	5 90	0 78	5 0
		63	11	—	—	—	—	″	″	1 034	14 63	3 13	2 74		0 39	5 51	5 12	0 87	4.4
		87	15	—	—	—	—	″	″	1 034	15 31	4 11	3 44		0 67	4 55	5 84	0 81	5 5
		111	19	—	—	—	—	″	″	1 034	14 09	3 67	—		—	4 42	5 30	0 70	6 0
		135	23	—	—	—	—	″	″	1 035	14 34	4 41	3 80		0 61	4 03	5 17	0 73	6 5

TABLE 2—*Continued*

NUMBER OF EXPERIMENT	NUMBER OF PARTURITION	HOURS AFTER PARTURITION	NUMBER OF MILKING	COLOR			pH	ALCOHOL TEST	BOILING TEST	SPECIFIC GRAVITY	TOTAL SOLID	TOTAL PROTEIN	CASEIN	LACTO-GLOBULIN	ALBUMIN	FAT	LACTOSE	ASH	MILK QUANTITY
				Red	Yellow	Blue					*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*
X	6	Immediately after	1	1.2	1 2	0	6 61	+	+	1 0695	23 12	19 46	7 40		12 06	3 52	1 28	0.86	16.2
		15	3	1 0	1 2	0	6 00	+	+	1 0482	21 23	11 68	5 79		5 89	5 98	2 75	0 82	1.0
		39	7	0 6	0 5	0 6	6 17	+	—	1 0340	13 67	4 06	3 28		0 78	—	—	0 73	9.0
		63	11	0.6	0 4	0 7	6 26	—	″	1 0317	15.07	3 91	1 99		1 92	5 31	5 11	0.74	12.0
		87	15	0 5	0 4	0 7	5 74	″	″	1 0334	15 35	3 86	3 00		0 86	6 46	4 28	0.75	15.0
		111	19	—	—	—	—	″	″	1 0350	15 05	4 32	3 64		0 68	5 84	4 14	0 75	20.0
XI	1	Immediately after	1	—	—	—	—	+	+	1 044	21 62	11 54	5 86	5 68		6 92	2 23	0.93	2.0
		28	6	—	—	—	—	+	—	1 043	16 68	6 85	3 02	3 83		5 06	3 83	0 94	6.6
		52	10	—	—	—	—	—	″	1 034	13 13	3 17	—	—		—	—	0 72	9.3
		76	14	—	—	—	—	″	″	1.034	14 57	5 05	3 92	0.12		5 55	4 22	0.75	10.3
		100	18	—	—	—	—	″	″	1 033	13 70	3 75	2 87	0 88		—	—	0 81	9.0
		124	22	—	—	—	—	″	″	1 036	14 01	3 76	3 17	0 58		—	—	0 73	9.6
		148	26	—	—	—	—	″	″	1 034	15 25	3 81	3 20	0 62		6 01	4.70	0 73	14.0
		172	29	—	—	—	—	″	″	1.032	15 70	3 78	3 19	0 59		—	—	0 65	13.7
		196	32	—	—	—	—	″	″	1 037	14 61	4 00	3 46	0 54		—	—	0.75	16.6
		220	36	—	—	—	—	″	″	1 033	14 80	3 77	3 03	0 74		6 39	3.93	0.71	13.4
XII	8	Immediately after	1	—	—	—	—	+	+	1 065	21 89	15 73	6.17		10 56	3 92	1 26	0.98	5.7
		26	6	—	—	—	—	+	—	1 055	19.85	8 68	—		—	—	—	1 01	5.7
		50	10	—	—	—	—	+	″	1 040	15.02	4 89	3 85		1.04	5 37	3.78	0.98	8.5
		74	14	—	—	—	—	—	″	1 035	14 30	4.13	3 33		0 80	5 73	3 63	0.81	7 5
		98	18	—	—	—	—	″	″	1.031	14 36	3 64	3 13		0 51	5.11	4.82	0 79	8 5
		112	22	—	—	—	—	″	″	1 033	13 55	4 52	—		—	—	—	0.76	10.1
		136	26	—	—	—	—	″	″	1 034	14 24	3 48	3.00		0.48	6.11	3.90	0 75	11.0

XIII	1	Immediately after	1	—	—	—	—	+	+	1 0567	21 28	14 18	6 54	7 64	3 50	2 56	1 04	12 4
		15	3	—	—	—	—	+	—	1 036	15 48	6 34	4 98	1 36	4 44	3 80	0 90	3.7
		39	7	—	—	—	—	+	"	1 032	13 82	4 83	3 65	1 18	—	—	0 81	8 0
		63	11	—	—	—	—	—	"	1 035	13 08	4 69	3 85	0 84	4 81	2 70	0 88	6.5
		87	15	—	—	—	—	"	"	1 034	13 50	4 38	3 95	0 43	5 84	2 54	0 74	8.5
		111	19	—	—	—	—	"	"	1 035	13 62	3 90	3 40	0 50	5 79	3.14	0 76	12 0
		135	23	—	—	—	—	"	"	1 0325	12 80	3 48	3 97	0 51	4 59	4 03	0 70	11 0
		159	27	—	—	—	—	"	"	1 032	13 54	3 41	2 84	0 57	4 62	4 79	0 72	8.7
XIV	6	Immediately after	1	—	—	—	—	+	—	1 0375	17 36	10 84	4 57	6 27	3 58	1 94	1 00	3 2
		17	3	—	—	—	—	+	"	1 033	14 74	5 99	5 47	0 52	5 13	2 76	0 86	6 3
		41	7	—	—	—	—	+	"	1 034	15 30	4 75	4 45	0 30	5 87	3 84	0 84	10 5
		65	11	—	—	—	—	—	"	1 033	16 00	3 50	3.24	0 26	—	—	0 81	10 0
		89	15	—	—	—	—	"	"	1 033	15 20	3 95	3 49	0 46	—	—	0 68	13 0
		113	19	—	—	—	—	"	"	1 032	14 56	3 30	2 99	0 31	—	—	0 72	10 7
		137	23	—	—	—	—	"	"	1 032	14 34	3 38	2 61	0 77	—	—	0 78	7.4
XV	9	Immediately after	1	—	—	—	—	+	+	1 0567	22 20	14 65	—	—	—	—	0.91	4.5
		27	5	—	—	—	—	+	—	1 0358	17 98	7 48	4 24	3 24	—	—	0 81	1 5
		51	9	—	—	—	—	+	"	1 0320	16.70	4 77	3 92	0 85	—	—	0.74	2 6
		75	13	—	—	—	—	—	"	1 034	13 42	4 16	3 64	0 52	—	—	0 70	3.7
		99	17	—	—	—	—	"	"	1 032	12 82	4 25	3 50	0 75	—	—	0 72	3 7
		128	21	—	—	—	—	"	"	1 033	13 36	3 51	3 07	0 44	—	—	0 78	4 0
		147	26	—	—	—	—	"	"	1 031	13 78	3 33	3 01	0 31	—	—	0.71	4 8
XVI	1	Immediately after	1	0 3	0 3	0 3	6 14	+	+	1 059	18 92	11 73	5 11	6 62	3 71	2 50	0 98	14 0
		16	6	0 3	0 3	0 3	6 24	+	—	1 0485	16 94	5 63	4 69	0 94	5 89	4 56	0 86	10 5
		40	10	0 3	0 3	0 3	6 33	+	"	1 038	14 70	4 35	3 93	0 42	6 67	2 80	0 88	10 5
		64	14	0 3	0 1	0 5	6 34	—	"	1 0345	13 90	4 03	3 30	0 73	—	—	0 80	11 6
		88	18	0 3	0 1	0 5	6 32	"	"	1 0325	14 06	3 29	3 05	0 24	5 36	4 61	0 80	12 0
		112	22	0 4	0 1	0 6	6 35	"	"	1 034	13 64	3 55	2 56	0 79	6 12	3 18	0 79	13 8
		136	26	0 3	0 1	0 6	6 35	"	"	1 0325	13 84	3 44	2 68	0 76	4 44	5 20	0 76	15 6

TABLE 2—*Concluded*

NUMBER OF EXPERIMENT	NUMBER OF PARTURITION	HOURS AFTER PARTURITION	NUMBER OF MILKING	COLOR			pH	ALCOHOL TEST	BOILING TEST	SPECIFIC GRAVITY	TOTAL SOLID	TOTAL PROTEIN	CASEIN	LACTO-GLOBULIN	ALBUMIN	FAT	LACTOSE	ASH	MILK QUANTITY
				Red	Yellow	Blue													
											per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*
XVII	6	Immediately after	1	—	—	—	—	+	+	1 059	21 56	12 06	—		—	6 69	1.92	0 89	4 0
		26	6	—	—	—	—	+	—	1 038	15 04	5 33	—		—	4 52	4 37	0 82	1 0
		50	10	—	—	—	—	+	〃	1 037	14 00	3 96	—		—	4 46	4.73	0.85	1 0
		74	14	—	—	—	—	+	〃	1 036	14 40	4 48	—		—	4 50	4.57	0.85	1 5
		98	18	—	—	—	—	—	〃	1 036	—	—	—		—	—	—	—	2 3
		122	22	—	—	—	—	〃	〃	—	—	—	—		—	—	—	—	2.4
XVIII	9	Immediately after	1	0 7	0 4	0	5 01	+	—	—	—	—	—		—	—	—	—	10.5
		32	6	1 3	0 2	0	6 05	+	〃	1 034	13 65	3 94	—		—	4 89	3.91	0.92	4.0
		56	10	1 0	0 7	0	6 58	—	〃	1 0324	13 25	4 10	—		—	4 66	3 85	0 63	6.2
		80	14	0 8	0 4	0 5	6 56	〃	〃	1 0328	13 96	4 68	—		—	4 73	3 95	0.63	5.5
		104	18	—	—	—	—	〃	〃	1 033	—	—	—		—	—	—	—	6.2
		128	22	—	—	—	—	〃	〃	1 032	—	—	—		—	—	—	—	6.2

TABLE 3

The presence of certain agglutinins in normal bovine colostrum

NUMBER OF EXPERIMENT	COW		SAMPLE		ORGANISMS USED AS ANTIGENS IN TESTING FOR AGGLUTININS IN COLOSTRUM				
	Breed	Number of parturition	Hours after parturition	Number of milking	*Bact. coli*	*Bact. paratyphosum* B	*Bacillus abortus*	*Bacillus melitensis*	*Vibrio fetus*
1	Ayrshire 94	4	0	1	640	80	20	40	
			4	2	640	160	20	—	
			12	3	160	80	10	—	
			24	4	80	40	—	—	
			29	5	40	40			
			34	6	20	10			
			48	7	20	20			
			53	8	10	—			
			58	9	—	—			
			72	10	—	—			
			77	11	—	10			
			82	12	—	—			
			96	13	—	—			
				14		—			
2	Ayrshire 94	5	19*		160	40	10	40	320
			0	1	320	40	80	40	1,280
			9	2	80	40	20	40	80
			33	3	10	10	10	—	20
			48	4	—	—	—	10	20
			51	5	—			—	—
			57	6	—				—
3	Ayrshire 86	6	18	3	160	40	20	20	320
			33	4	80	40	—	—	640
			37	5	40	20	—	—	80
			57	6	10	—			40
			61	7	—				20
			66	8					10
			81	9					10
			85	10					10
			90	11					—
4	Ayrshire 115	1	12	3	640	80	80	320	1,280
			39	4	80	20	20	40	80
			43	5	20	—	—	—	160
			63	6	10	—	—	—	80
			67	7	—				40
			72	8					20
			76	9					10
			96	10					—
			106	11					—

* This milk was drawn 19 hours before parturition.

TABLE 3—*Continued*

NUMBER OF EXPERIMENT	COW		SAMPLE		ORGANISMS USED AS ANTIGENS IN TESTING FOR AGGLUTININS IN COLOSTRUM				
	Breed	Number of parturition	Hours after parturition	Number of milking	*Bact. coli*	*Bact. paratyphosum* B	*Bacillus abortus*	*Bacillus melitensis*	*Vibrio fetus*
5	Ayrshire 110	2	0	1	160	40	160	80	640
			4	2	80	40	80	80	160
			10	3	40	10	10	10	20
			24	4	20	10	10	10	40
			28	5	—	—	—	—	20
			34	6	—				10
			48	7					10
			52	8					—
6	Ayrshire 116	1	0	1	640	160	40	80	2,560
			17	2	160	80	10	20	320
			21	3	40	20	10	20	160
			34	4	20	—	—	—	40
			39	5	10	—			20
			44	6	10	—			20
			57	7	—	—			10
			62	8					10
			67	9					—
7	Guernsey 173	5	0	1	20	10	—	—	
			24	6	40	—	10	—	
			48	10	—	—	—		
			72	14	—				
8	Guernsey 168	8	0	1	640	80	20	40	160
			25	6	10	—	—	10	10
			49	10	—			10	10
			73	14	10			—	10
			97	18	10				10
9	Guernsey 189	2	15	3	10	—	—	—	20
			39	7	—	—	—	—	20
			63	11	—				10
			87	15	—				10
			111	19					—
10	Holstein 195	6	0	1	40	10	40		—
			15	3	10	—	10		—
			39	7	—		—		
			63	11					
			87	15					

TABLE 3—*Concluded*

NUMBER OF EXPERIMENT	COW		SAMPLE		ORGANISMS USED AS ANTIGENS IN TESTING FOR AGGLUTININS IN COLOSTRUM				
	Breed	Number of parturition	Hours after parturition	Number of milking	*Bact. coli*	*Bact. paratyphosum* B	*Bacillus abortus*	*Bacillus melitensis*	*Vibrio fetus*
11	Holstein 237	1	0	1	160	—	20		—
			28	6	80	—	10		—
			52	10	20		—		
			76	14	10				
			100	18	—				
12	Holstein 171	8	0	1	160	20	40	40	640
			26	6	160	10	40	20	160
			50	10	—	—	10	10	10
			74	14	—		—	—	10
			98	18				—	—
			112	22					—
13	Holstein 243	1	0	1	1,280	20	20	40	640
			15	3	40	10	—	—	40
			39	7	—	—		—	—
			63	11		—			20
			87	15					—
14	Holstein 197	6	0	1	10	—	—	—	—
			17	3	—	—	—	—	—
15	Holstein 173	9	0	1	160	80	20	20	320
			27	5	40	20	10	10	80
			51	9	10	—	—	—	10
			75	13	10	—			10
			99	17	—				—
			128	21	—				—
16	Holstein 239	1	0	1	160	20	20	20	320
			16	6	10	—	—	—	40
			40	10	—				—
			64	18					—
									—
17	Guernsey 170	6	0	1	40	20	—	10	
			26	6	80	10	—	20	
			50	10	80	20	10	40	
			74	14	80	—	10	20	
18	Holstein 169	9	0	1	—	—	—	—	
			32	6	—	—	—	—	
Control	Rennet				—	—	—	—	—

immunized with horse erythrocytes, the serum being made inactive as the complement, fresh serum (0.05 cc.) of guinea-pig was used. The hemolysin-titre of the rabbit serum and its complement demonstrating power are indicated in the following table:

Hemolysin-titre

	DILUTION OF SERUM								
	50	100	200	400	800	1600	3200	6400	12800
Result of hemolysis	+++	+++	+++	+++	+++	++	+	±	–

The complement demonstrating power

A survey was made as to the existence or non-existence of hemolysis by mixing suitable quantity of fresh serum of cow.

	AMOUNT OF COMPLEMENT IN COW					
	0.05 cc	0.01 cc	0.005 cc	0.001 cc.	0.0005 cc.	0.0001 cc.
Result of hemolysis	+++	+++	+++	+	–	–

Two-tenths cubic centimeter of amboceptor diluted 40 times and 1 cc. of 1 per cent suspension of horse erythrocytes (total quantity 2.2 cc.) were used for the purpose.

In order to determine the presence of complement in colostrum, different quantities of colostrum (1.0, 0.8, 0.6, 0.4 and 0.2 cc., respectively) were put separately into fine test tubes. Proper quantities of physiological salt solution were then added to the colostrum contained in each tube, the quantity of the contents of each tube being made uniform at 1 cc. and again to the contents of each tube was added 0.2 cc. each of immune serum or amboceptor diluted 40 times (dilution was resorted to each time immune serum was used for similar purposes). Adding to the mixture 1 cc. suspension of horse erythrocytes and completely mixing the entire contents, the mixture was incubated at 37°C. for 1 hour, and after keeping in an ice-room for 15 hours the contents of each tube was examined. Throughout the examination the centrifugal separation process was carried out, constant care being taken to assure the precipitation of erythrocytes to the bottom of the tubes. For the purpose of contrast fresh serum of a guinea-pig and a mixture of physiological salt solution (1 cc.) and suspension of erythrocytes (1 cc.) were provided as the substitutes for the colostrum in order to prove the normality of immune serum and erythrocytes. (With the former the result of hemolysis was satisfactory, the case being the reverse with the latter.)

The above experiment was applied to all of the colostrum put to test, the result, however, proving negative throughout, whereas with the addition of fresh serum (0.05 cc.) extracted from a guinea-pig to the contents of each tube the result of hemolysis was complete.

RESULTS

The results are summarized hereunder:

1. In the normal parturition, the colostrum always contains agglutinins.
2. The period of the presence of agglutinins in the colostra ranges from 1 to 4 days after parturition.
3. Agglutinins are contained in the colostra principally after parturition; but, as a rule, they speedily decrease in number afterward and finally disappear.
4. Generally, agglutinins reacting with *Bacterium coli* and *Vibrio fetus* are found to be most abundant, those reacting with *Bact. paratyphosum B*, *Bacillus melitensis* and *Bacillus abortus* coming next in the named order.
5. The breeds of cattle and the number of parturition apparently have nothing to do with the quantity of agglutinins and the length of the period of their existence in the colostra after parturition.
6. In the colostrum obtained from a cow suffering from the inflammation of the udder there seems to be no fixed rule as to the appearance of agglutinins.
7. In the milk secreted by a cow which aborted in the fourth month after pregnancy, the presence of agglutinins has not been proved.
8. A large quantity of agglutinins was found in the milk secreted 19 hours before parturition.

9. Throughout the series of experiments made by the authors with fresh and normal colostra the presence of complements were not proved.

10. Colostra of the same and of different cows are exceedingly variable in chemical composition. The specific gravity and total ash are fairly constant. There were wide fluctuations in the hydrogen ion concentration, color, coagulation, the percentage of total proteins and the relative proportions of each, the percentage of lactose, and the fat content.

REFERENCES

(1) BERTARELLI, E.: Über aktive und passive Immunisation der Neugeborenen und Sauglinge auf dem Wege der Verdaungsorgane. Centralbl. f. Bakt., etc., I Abt., Org., 1905, xxxix, 285–303.

(2) FAMULENER, L. W.: On the transmission of immunity from mother to off spring. A study upon serum haemolysins in goats. J. Infekt. Dis., 1912, x, 332–368.

(3) v. ZUBRZYCKI, J., UND WOLFSGRUBER, R.: Normale Hämoagglutinine in der Frauenmilch und ihr Ubergang auf das Kind. Deutsche med. Wochenschr., 1913, xxxix, 210–212.

(4) REYMANN, G. C.: On the transfer of the so-called normal antibodies from mother to mother to offspring. Jour. Immunol., 1920, v, 227–238.

(5) HOWE, P. E.: The differential precipitation of the proteins colostrum and a method for the determination of the proteins in colostrum. Jour. Biol. Chem., 1922, ii, 51–68.

(6) LEWIS, J. H., AND WELLS, H. G.: The functions of the colostrum. Jour. Amer. Med. Assoc., 1922, lxxviii, 863–865.

(7) LITTLE, R. B., AND ORCUTT, M. L.: The transmission of agglutinins of *Bacillus abortus* from cow to calf in the colostrum. Jour. Exp. Med., 1922, xxxv, 161–171.

(8) SMITH, TH., AND LITTLE, R. B.: The significance of colostrum to the new-born calf. Jour. Exp. Med., 1922, xxxvi, 181–198.

(9) SMITH, TH., AND LITTLE, R.B.: The absorption of specific agglutinins in homologous serum fed to claves during the early hours of life. Jour. Exp. Med., 1923, xxxvii, 671–683.

CALCULATING RATIONS FOR DAIRY COWS*

T. E. WOODWARD

Bureau of Dairy Industry, United States Department of Agriculture

In many dairy-cow investigations, the animals must be fed according to some definite plan, if accurate comparisons are to be made of the production of dams and daughters and the effect of various feeds over entire lactation periods. The usual method of feeding is to select some standard, and then to provide nutrients in such amounts as will meet the requirements of this standard. In some experiments, a little more than the required amount is fed to make sure that the animal receives sufficient nutrients for maintenance and milk production.

Because of the amount of work involved in calculating nutrient requirements for large numbers of animals, a quick and easy method of figuring rations was needed at the Bureau of Dairy Industry Experiment Farm at Beltsville, Maryland. After some study and the trying out of different methods, a system was developed (table 1) by which the rations could be easily figured at the rate of one a minute, if the weight of the cow and the quantity and quality of milk produced were known. These rations, when compared with those obtained by exact calculations, are ordinarily accurate to within one pound of grain per cow per day. This method has been used successfully at Beltsville for several years. It is now passed along with the thought that it may be of some value to other investigators. Although the system given has been prepared to fulfill the requirements of the Savage standard as to total digestible nutrients, there is no reason why a similar method can not be used for any standard desired.

The feeds generally used at Beltsville are No. 2 alfalfa hay, good corn silage, dried beet pulp, and two grain mixtures. The total digestible nutrients of the feeds, according to Henry and Morrison's "Feeds and Feeding," are as follows: beet pulp, 71.6 per

---

* **Received for publication August 6, 1930.**

TABLE 1

Number of pounds alfalfa hay required daily (Savage Standard) for cows producing different quantities of milk varying in butterfat test

MILK	BUTTERFAT TEST—PER CENT																				
	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0
lbs.	*lbs.*	*lbs.*	*lbs*	*lbs*	*lbs.*	*lbs.*	*lbs*	*lbs.*	*lbs*	*lbs*	*lbs*	*lbs.*	*lbs*	*lbs.*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*	*lbs*
10	6	6	6	6	7	7	7	7	7	8	8	8	8	8	9	9	9	9	9	10	10
11	6	6	7	7	7	7	8	8	8	8	9	9	9	9	10	10	10	10	10	11	11
12	7	7	7	8	8	8	8	9	9	9	9	10	10	10	10	11	11	11	11	12	12
13	7	7	8	8	8	8	9	9	9	10	10	10	11	11	11	12	12	12	12	13	13
14	8	8	8	9	9	9	10	10	10	11	11	11	12	12	12	12	13	13	13	14	14
15	8	9	9	9	10	10	10	11	11	11	12	12	12	13	13	13	14	14	14	15	15
16	9	9	10	10	10	11	11	11	12	12	12	13	13	13	14	14	14	15	15	15	16
17	9	10	10	10	11	11	12	12	12	13	13	13	14	14	15	15	15	16	16	16	17
18	10	10	11	11	12	12	13	13	13	14	14	14	15	15	16	16	16	17	17	17	18
19	11	11	11	12	12	13	13	14	14	14	15	15	15	16	16	17	17	18	18	18	19
20	11	12	12	13	13	14	14	15	15	15	16	16	16	17	17	18	18	19	19	19	20
21	12	12	13	13	14	14	15	15	15	16	16	17	17	18	18	19	19	19	20	20	21
22	12	13	13	14	14	15	15	16	16	17	17	18	18	19	19	19	20	20	21	21	22
23	13	13	14	15	15	16	16	17	17	18	18	18	19	19	20	20	21	21	22	22	23
24	13	14	14	15	16	16	17	17	18	18	19	19	20	20	21	21	22	22	23	23	24
25	14	14	15	16	16	17	18	18	19	19	20	20	21	21	22	22	23	23	24	24	25
26	14	15	16	16	17	17	18	19	19	20	20	21	21	22	23	23	24	24	25	25	26
27	15	16	16	17	18	18	19	20	20	21	21	22	22	23	23	24	24	25	26	26	27
28	16	16	17	18	18	19	19	20	20	21	22	22	23	23	24	25	25	26	26	27	28
29	16	17	17	18	19	20	20	21	21	22	23	23	24	24	25	26	26	27	28	28	29
30	17	17	18	19	20	20	21	22	22	23	24	24	25	25	26	27	27	28	28	29	30
32	18	18	19	20	21	22	22	23	24	24	25	26	26	27	28	28	29	30	30	31	32
34	19	20	21	21	22	23	24	25	25	26	27	27	28	29	29	30	31	31	32	33	33
36	20	21	22	23	24	24	25	26	27	28	28	29	30	30	31	32	33	33	34	35	35
38	21	22	23	24	25	26	27	28	28	29	30	31	31	32	33	34	34	35	36	37	37
40	22	23	24	25	26	27	28	29	30	31	31	32	33	34	35	35	36	37	38	39	39
42	23	24	25	26	27	28	29	30	31	32	33	33	34	35	36	37	38	39	40	41	41
44	24	25	27	28	29	30	31	32	33	34	35	35	36	37	38	39	40	41	42	43	43
46	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	44	45
48	27	28	29	30	31	33	34	35	36	37	38	39	40	41	41	43	44	44	45	46	47
50	28	29	30	31	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
52	29	30	31	33	34	35	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
54	30	31	33	34	35	37	38	39	40	41	42	43	44	46	47	48	49	50	51	52	
56	31	32	34	35	37	38	39	41	42	43	44	45	46	47	48	50	51	52	53		
58	32	34	35	36	38	39	41	42	43	44	46	47	48	49	50	51	53	54			
60	33	35	36	38	39	41	42	43	45	46	47	48	49	51	52	53	54				
64	36	37	39	40	42	43	45	46	48	49	50	51	53	54	55	57					
68	38	40	41	43	44	46	48	49	50	52	53	55	56	57	59						
72	40	42	43	45	47	49	51	52	53	55	57	58	59	61							
76	42	44	46	48	50	51	53	55	57	58	60	61	63								
80	44	46	48	50	52	54	56	58	60	61	63										
84	47	49	51	53	54	57	59	61	62	64											
88	49	51	53	55	56	60	62	64	65												
92	51	53	55	57	59	63	65	66													
96	54	56	58	60	62	66	67														
100	56	58	60	63	65	68															

TABLE 1—*Continued*

	Weight	*Hay*
For maintenance	700	11
	800	12
	900	14
	1,000	15
	1,100	17
	1,200	18
	1,300	20
	1,400	21
	1,500	23

cent; corn silage, 17.7; and alfalfa hay, 51.6. From Henry and Morrison, also, it is estimated that the grain mixtures contained 73.1 and 73.7 per cent digestible nutrients. In total digestible nutrients, 1 pound of alfalfa is equal to about 0.7 pound of grain or beet pulp, or to about 3 pounds of silage.

The following examples show how the information contained in table 1 is used:

Example 1. Daily ration for cow weighing 1,500 pounds and giving 50 pounds milk testing 3 per cent butterfat

Requirements in terms of hay:	
Maintenance	23 pounds
Milk	28 pounds
Total	51 pounds
Roughage received:	
Silage	45 pounds, equivalent to 15 pounds hay
Hay	18 pounds
Total	33 pounds

51 − 33 = 18 pounds hay, the equivalent of which is to be made up of grain

18 pounds × 0.7 = 12.6 pounds, the quantity of grain required. The ration, then, is 12.6 pounds of grain, 18 pounds of hay, and 45 pounds of silage. The exact requirements, according to the Savage standard, when the analyses stated in "Feeds and Feeding" are used, are 26.24 pounds digestible nutrients; the ration given contains 26.54 pounds.

Example 2. Daily ration for cow weighing 1,100 pounds and giving 20 pounds of milk testing 4 per cent butterfat

Requirements in terms of hay:	
Maintenance	**17 pounds**
Milk	**14 pounds**
Total	**31 pounds**

Roughage received:

Silage	33 pounds, equivalent to 11 pounds hay
Hay	14 pounds
Total	25 pounds

31 − 25 = 6 pounds to be made up in grain.
6 × 0.7 = 4.2 pounds, the amount of grain given.
Nutrients required, 15.72 pounds; nutrients in ration given, 16.16 pounds.

Example 3. Daily ration for cow weighing 800 pounds and giving 30 pounds of milk testing 5 per cent butterfat

Requirements in terms of hay:

Maintenance	12 pounds
Milk	24 pounds
Total	36 pounds

Roughage received:

Silage	24 pounds, equivalent to 8 pounds hay
Hay	10 pounds
Total	18 pounds

36 − 18 = 18 pounds to be made up in grain.
18 × 0.7 = 12.6 pounds, the amount of grain needed to complete the ration.
If 4 pounds beet pulp are fed, this will leave 8.6 pounds grain. Nutrients required, 18.49 pounds; nutrients in ration given, 18.61 pounds.

In the three examples shown above, the nutrients given exceeded those required by 0.3 pound, on an average. This is no more than would be needed for the production of 1 pound of milk.

The method is thought to be accurate enough for investigations made with feed for which no special chemical analyses are given. No doubt the variation in composition of feeds results in a larger error than does the use of this method.

THE FEATHERING OF EVAPORATED MILK IN HOT COFFEE*

RANDALL WHITAKER

Research Laboratories, National Dairy Products Corporation Baltimore, Maryland

Milk, cream, and evaporated milk occasionally curdle or "feather" when placed in hot beverages such as coffee. The term "feather" is descriptive of the fluffy, feather-like appearance of the curd particles which form under certain conditions. The subject of the occasional feathering of evaporated milk in hot coffee has received practically no attention by scientific workers, as indicated by the absence of published experimental work. Hunziker (4) recognizes the feathering of evaporated milk but does not include it as a defect of this product. He states, while discussing the manufacture of this product, that feathering is usually associated with high viscosity and the use of excessive homogenization pressures. He also reports that the use of an homogenizer with a spring controlled valve induces feathering.

The feathering of cream in hot coffee has been studied by several investigators (1), (3), (7), (8). It has been shown that acidity of the cream, extent of homogenization, heat treatment, fat content, extent of fat globule clumping, salt balance, method of adding the cream to the coffee and the kind of water used in preparing the coffee influence the feathering of cream in coffee. Evaporated milk is used in much the same manner as cream. This product, although containing relatively more solids-not-fat and less fat, behaves in coffee quite similar to fresh cream in some respects.

In this paper is recorded the observations made during an investigation of the causes of and conditions necessary for the feathering of evaporated milk in hot coffee.

COAGULATION OF EVAPORATED MILK IN COFFEE

On account of their protein content, fluid dairy products are susceptible to coagulation by heat. The temperature at which

---

* Received for publication August 16, 1930.

coffee is served is sufficiently high to occasionally cause partial coagulation of the milk, cream, evaporated milk, etc. used in it. Under certain conditions, there results well defined curd particles which give rise to the phenomena known as feathering.

Because of the relatively greater concentration of the coagulable protein in evaporated milk, the feathering of this product is more noticeable and generally more pronounced than is the feathering of milk and cream. Heat coagulation of milk has been studied by Sommer and Hart (6). The salt balance, which may be briefly described as the relative amounts of available calcium and magnesium as compared with the phosphates and citrates, has been shown to be of paramount importance. These workers contend that when the calcium and magnesium is high, the milk can be stabilized by the addition of citrates and phosphates. In like manner adding soluble calcium and magnesium will stabilize milks which are high in citrates and phosphates. Doan (2) and Tracy and Ruehe (7) have supplied proof that the decreased stability resulting from homogenization is caused by an increase in calcium and magnesium resulting from the absorption of the phosphates, citrates, and casein on the increased surface presented by the homogenized fat. In like manner the decreased stability noted with high acidity, is attributable to the increased availability of the divalent cations in the acid reaction.

It is contended by some workers (5) that there are other factors besides the salt balance such as acidity, which are concerned with heat coagulation. It may be said, however, that in practically every case, it is possible to alter the heat stability of a milk by effecting a change in the salt balance.

Using the salt balance theory as a basis it may be predicted that any factor which tends to increase the amount of available calcium will result in increased feathering. This may be brought about by either directly increasing the available calcium or by removing such anions as phosphate, citrate, etc. which keep calcium in an unavailable form. The addition of such salts as sodium citrate and secondary sodium phosphate will reduce the available calcium and the tendency to feather.

THE FEATHERING TEST

Prior to making a study of the factors concerned with the feathering of evaporated milk in hot coffee, it was deemed advisable to have some means of measuring the susceptibility of a milk to feather in hot coffee. A feathering test was therefore devised. This test was based on the previously noted influence of the salt balance and was not dependent upon an acidity effect. If a milk failed to feather when placed in hot coffee, it was made to do so by the addition of certain salts; when feathering was observed, the addition of certain other salts would prevent it. By the addition of a known amount of the specific salt a measure of the susceptibility of the milk to feather was thus obtained.

The coffee used in this test was prepared by heating to the boiling point 250 cc. of distilled water and 10 grams of ground coffee and immediately straining through fine cheese cloth. The coffee thus prepared was kept at 95 to 100°C. until used.

When a sample of evaporated milk was to be tested, 5 cc. of milk was diluted with 1 cc. of distilled water. One cubic centimeter of this mixture was then rapidly added to 10 cc. of the hot coffee solution and immediately stirred. If the appearance of the coffee in the test tube was uniform throughout and there was no evidence of feathering, the test was repeated, diluting another 5 cc. portion of the milk with 1 cc. of a 1:9 solution of calcium chloride in place of the 1 cc. of distilled water. The 1:9 calcium chloride solution was prepared by mixing 1 cc. of N/2 $CaCl_2$ and 9 cc. of distilled water. If the coffee again failed to feather, the test was repeated using 1 cc. of a 2:8 $CaCl_2$ solution. This procedure was continued until feathering was noted. The number of cc. of N/2 $CaCl_2$ used in making the 10 cc. stock solution was taken as the unit of the test. The result of the test was then expressed as the number of these units which first caused feathering.

If the milk was found to feather without the addition of $CaCl_2$ then N/2 sodium citrate was used. Increasing amounts were added in the manner described above until feathering failed to appear.

To distinguish between the calcium chloride units and the sodium citrate units, a minus sign (−) was prefixed to the calcium chloride values, denoting that the milk did not feather normally, while a positive sign (+) was prefixed to the citrate values to indicate a positive feathering test. Table 1 illustrates several selected examples. This test is a measure of the susceptibility of a given sample of evaporated milk to feather. As indicated in table 1, milks having minus values do not feather, while positive values show this defect. The extent to which a given sample does or does not feather is indicated by the magnitude of the number given. For uniform and reproducible results the procedure as outlined must be adhered to rigidly.

TABLE 1

Illustration of feathering test values

FEATHERING VALUES		STOCK SOLUTION		
		N/2 salt solution	Distilled water	
		parts	*parts*	
−3	Feathering produced by 1 cc. of	3 +	7	Calcium chloride
−2		2 +	8	
−1		1 +	9	
+1	Feathering produced by 1 cc. of	1 +	9	Sodium citrate
+2		2 +	8	
+3		3 +	7	

FACTORS WHICH INFLUENCE FEATHERING

Experimental work revealed several factors which influenced the extent to which evaporated milk feathered when placed in hot coffee. These factors, with a brief discussion, are as follows:

1. *Salt balance of the milk.* As previously pointed out a milk which has an excess of available calcium and magnesium will tend to feather when placed in hot coffee. An excess of calcium may be due to the natural salt content of the milk, to the addition of a calcium neutralizer before condensing, to high acidity, to depletion of opposing anions on homogenization, etc. The effect of

excess calcium was demonstrated in this work by the fact that with every sample of milk examined, the addition of a soluble calcium salt, such as the chloride, produced increased feathering whereas the tendency to feather was reduced when the available calcium was removed from the sphere of action by the addition of such substances as secondary sodium phosphate, sodium citrate, sodium hydroxide, etc. Evaporated milk which was unstable due to excessive amounts of citrates and phosphates was not encountered. This form of unstability is very infrequent.

2. *Hardness of the water.* Tracy and Ruehe (7) found that the hardness of the water used in preparing coffee had an influence on the feathering of cream. It has been shown that calcium (and magnesium) in solution tends to induce feathering in evaporated milk. In table 2 is shown the results of a feathering test performed on coffees made from samples of water, procured from six different cities. A single source of evaporated milk was used in this work. The hardness of each sample of water was estimated by means of its soap consuming power. It will be seen from this table that there was insufficient calcium in the waters collected to produce a change in the feathering value of the sample of milk used in this test.

TABLE 2

The influence of the hardness of the water used in the preparation of the coffee on the feathering of evaporated milk

SOURCE OF WATER	HYDROGEN ION CONCENTRATION	HARDNESS OF WATER	FEATHERING TEST (1 CC IN 10 CC)
	pH	*p p m* *	
Distilled	6.9	0	−4
Baltimore, Md	6.8	35	−3
Madison, Wis.	7.6	35	−4
Chicago, Ill.	7.3	80	−4
Detroit, Mich.	6.8	60	−4
New York City	6.5	20	−3
Tempe, Arizona	7.3	150	−3
Youngstown, Ohio	7.4	150	−4

* Calculated as calcium carbonate.

3. *Concentration of the coffee.* Several people were requested

to indicate the quantity of ground coffee they recommended for making a given volume of coffee. The average ratio was 10 grams of ground coffee to 250 cc. of water. As little as 7 grams was recommended in one case, while one housewife used 13.5 grams for this amount of water. Investigation showed that evaporated milk tended to feather more readily in strong coffee than in weak. It seems probable that the salts and the tannic acid extracted from the coffee assist in the feathering of the evaporated milk.

4. *Method of preparing the coffee.* It has been suggested that substances extracted from coffee influence the extent of feathering. It was to be expected, therefore, that the method of preparing the coffee would also have an effect, but only in so far as the salts and tannic acid were extracted from the coffee grounds. Considering the amount of water-soluble ash as a measure of extraction, it will be seen from table 3 that coffee prepared by the drip or percolation method was less liable to cause feathering than was coffee made by boiling in the pot. The data show that prolonged contact of the coffee and the grounds increased the quantity of soluble ash and consequently tended to increase feathering. The hydrogen ion concentration of the coffee remained constant and was independent of the method of preparation.

TABLE 3

Ash content and hydrogen ion concentration of coffee prepared by different methods (10 grams ground coffee and 250 cc. distilled water)

METHOD OF PREPARATION	TREATMENT OF COFFEE	ASH	HYDROGEN ION CONCENTRATION
		per cent	*pH*
Pot	Filtered immediately on boiling	0 13	4 93
	Filtered after standing 5 minutes	0 16	4 92
	Ditto, after 10 minutes	0 18	4 93
Percolated	Five minutes	0 14	4 92
	Ten minutes	0 14	4 95

5. *Amount of evaporated milk used in coffee.* Several individuals

were requested to indicate the quantity of evaporated milk preferred in a cup of coffee of standard size. In table 4 will be found the quantities indicated when the milk was placed in the cup prior to adding the coffee, and when the quantity of milk selected was dependent upon the color produced in the coffee by the milk. The inconsistencies noted made the average quantity of evaporated milk used in coffee somewhat uncertain. For all practical purposes, however, it was assumed that one tenth as much milk as coffee was the average amount used.

TABLE 4

Quantity of evaporated milk used in a cup of coffee of standard size (150 cc) by 11 individuals (10 grams ground coffee and 250 cc. water)

INDIVIDUAL	MILK ADDED PRIOR TO COFFEE	MILK ADDED TO COFFEE
	cc.	*cc*
A	13	10
B	16	7
C	8	17
D	11	5
EQ	6	9
F	20	
G	19	
H	7	5
I	20	15
J		13
K		10
Average .	13 3	10 1

Laboratory experiments indicated that feathering was always more pronounced when the smaller quantities of milk were used in the coffee. Better feathering conditions existed when a small amount of evaporated milk was added, because (*a*) under such conditions a relatively small amount of coagulable protein came in contact with a large quantity of acid, salt-containing coffee solution, (*b*) the resulting white curd particles were more noticeable against the darker background of the coffee and (*c*) the small quantity of evaporated milk was heated to a higher temperature than a larger volume would have been. From table 5 it will be seen that the pH of coffee-evaporated milk mixtures varies with the amount of milk used in a given quantity of coffee. When small portions of milk are used, the casein of the evaporated milk is brought near the isoelectric point and in consequence, coagulation takes place readily. It is probable, therefore, that individuals using small quantities of evaporated milk in coffee will encounter feathering more frequently than those who use large quantities of milk.

6. *Method of mixing milk and coffee.* Laboratory tests showed that feathering was more frequent when the evaporated milk was added slowly, drop by drop, to the hot coffee. Adding the milk in such a manner without stirring created conditions which foster coagulation for the reasons given under (5). In some cases, agitation destroyed the curdled appearance, in others the effect was permanent. When feathering presisted on agitation the curd particles often lost their fluffiness and became grainy in appearance. The tendency to feather was less if the coffee was added to the milk than if the milk was poured into the hot coffee. The use of sugar in quantities normally used had no effect on the feathering of evaporated milk in coffee.

TABLE 5

Hydrogen ion concentration of various coffee-evaporated milk mixtures

COMPOSITION OF COFFEE-MILK MIXTURE				HYDROGEN ION CONCENTRATION
Amount of coffee	Amount of milk	One cc of citrate solution	One cc of CaCl$_2$ solution	
cc	*cc*			*pH*
10	0.0			4.9
10	0.1			5.1
10	0.5			5.4
10	0.7			5.6
10	1.0			5.7
10	2.0			5.8
10	1.0	1:9		5.7
10	1.0	2:8		5.7
10	1.0	3:7		5.7
10	1.0		1:9	5.7
10	1.0		2:8	5.7
10	1.0		3:7	5.6

SUSCEPTIBILITY OF COMMERCIAL SAMPLES TO FEATHERING

Fifty-two cans of evaporated milk were tested for feathering in an attempt to estimate the extent to which the present day commercial product feathers in hot coffee. These cans, representing 25 different brands of evaporated milk, were purchased in stores in 7 cities throughout the United States. From table 6 it will be seen that only one sample was found to feather when 1 cc. of treated milk was tested in 10 cc. of coffee. The average feathering test value of all samples was −2.6. Some coffee drinkers prefer a smaller quantity of evaporated milk than was represented in these tests, and since feathering is more pronounced in such cases, some of the samples were tested by using 0.5 cc. of milk. When this small quantity of milk was used, milk feathering was encountered in about two thirds of the samples. However, the number of people using such a small quantity of milk is comparatively small. It seems probable that the average person using evaporated milk as now offered to the trade will encounter feathering but rarely when used in coffee which has been prepared in such a manner that it contains a minimum quantity of extracts such as tannic acid, salts, and the like.

In addition to the feathering test, other determinations were made on many of the samples. These determinations and the values obtained are included in table 6. An examination of the data in this table show that there is no apparent relationship between feathering as measured by the feathering test and any of the other physical constants that were determined.

From table 6, it will be seen that sample No. 1 was found to feather excessively. This sample was unusual in several respects. The milk was lumpy and possessed a coagulated appearance. It seemed probable that this sample was manufactured from milk of inferior quality, judging from the relatively high amino and ammonical nitrogen. Since the pH and the titratable acidity was normal, neutralization prior to condensing was indicated. The neutralization, probably accomplished with calcium and magnesium, decreased the heat stability of the milk and caused it to coagulate in the sterilizer. The excessive calcium-magnesium was likewise probably responsible for the marked feathering.

TABLE 6

Analysis of fifty-three samples of commercial evaporated milk

SAMPLE NUMBER	TOTAL SOLIDS	BUTTER FAT	ACIDITY	H ION CONCENTRATION	SPECIFIC GRAVITY	VISCOSITY	TOTAL N	AMMONIA N	AMINO N	FEATHERING TEST		PLATE COUNT	GENERAL REMARKS
										0.5 cc.	1.0 cc.		
	per cent	*per cent*	*per cent*	*pH*		*centipoises*	*per cent*	*per cent*	*per cent*			*cc.*	
1	27.4	8.4	0.37	6.00	1.056	43.0	0.99	0.010	0.048		+3	None	Coagulated, lumpy, flavor and odor normal
2	26.8	7.7	0.37	6.05	1.063	8.1	1.08	0.008	0.046		−1	Molds	Milk tasted thin and watery
3	28.5	7.9	0.35	6.03	1.062	8.3	1.08	0.007	0.048		−2	None	Fair quality
4	27.1	8.2	0.37	6.01	1.063	12.9	1.03	0.006	0.048		−2	None	Slight scorched flavor, color dark
4a										+2	−2		Good quality
4b										+1	−3		Good quality, crystals at bottom
4c										+1	−2		Good quality
5	25.4	7.3	0.31	6.04	1.056	6.1	1.00	0.006	0.037		−3	160	Rich flavor, fat churned slightly
6	28.4	8.1	0.35	6.08	1.069	11.2	1.05	0.005	0.046		−4	None	Fair flavor, fat churned
6a										−1	−4		Good quality
6b										−1	−3		Slight fat separation
6c										+1	−1		Fat churned and crystals at bottom
7	26.3	7.8	0.43	5.95	1.061	13.0	1.12	0.010	0.046		−4	None	Poor flavor, color dark, crystals
8	27.8	8.0	0.36	5.98	1.064	18.4	1.03	0.007	0.050		−3	None	Good quality, crystals at bottom
9	26.8	8.0	0.32	6.02	1.062	13.5	1.08	0.008	0.042		−3	Molds	Good quality
9a										−1	−3		Good quality
10	27.4	8.0	0.38	6.01	1.069	17.8	1.07	0.005	0.044		−4	None	Good quality
10a										+2	−2		Fair quality, crystals in milk
10b										+1	−2		Good quality, slight churning
10c										−1	−4		Good quality, crystals at bottom
10d										+1	−2		Good quality, slight churning
10e										+1	−2		Good quality, slight churning
10f										−1	−3		Good quality, slight churning

11	26 9	6 5	0 34	6 01	1 060	16 0	1 01	0 006	0 043		−4	None	Flavor scorched, crystals, dirty color
12	27 6	7 6	0 36	6 01	1 065	9 5	1 13	0 008	0 044		−4	None	Pungent flavor, slight churning
13	28 0	7 8	0 38	6 03	1.067	8 9	0 97	0 005	0 044		−2	820	Good quality
14	26 9	7 8	0 47	6 08	1 068	10 1	1 14	0 005	0 045		−3	None	Good quality
15	26 3	8 1	0.43	6 03	1 066	13 0	1 03	0 005	0 042		−2	None	Creamy flavor, light colored
16	27.0	7.6	0 43	6 03	1 067	11 7	1 11	0 005	0 046		−2	None	Bitter flavor, burned milk odor, churned
16a										+1	−3		Good quality
17	25.9	8.0	0 42	6 05	1 065	24 9	1 04	0 005	0 024		−3	None	Good quality, creamy
17a										−1	−2		Good quality
17b										−1	−3		Good quality
18	26 9	8 0	0 29	6 02	1 065	12 7	1 10	0 009	0 053		−3	None	Good quality, slight churning
19	25 8	7 8	0 30	6 04	1 064	9 1	1 00	0 004	0 042		−3	None	Good quality
19a										+2	−1		Good quality, crystals at bottom
20	26 4	7 8	0 34	6 04	1 066	9 7	1 10	0 010	0 040		−4	None	Poor quality, scorched, churned, pungent flavor
21	26 3	8 0	0 30	6 03	1 065	16 0	1 11	0 009	0 046		−3	None	Good quality, crystals at bottom
21a										+2	−2		Fair flavor, slightly churned
21b										−1	−3		Flavor flat, slight churning
21c										+2	−1		Good quality
21d										−1	−3		Good quality
21e										+1	−3		Good quality
21f										+2	−2		Good quality, crystals
21g										+1	−2		Good quality, crystals
22	25 6	8 0	0 34	6 01	1 063	19 1	1 11	0 007	0 041		−3	None	Creamy, dark color slightly churned
23	24 9	8 2	0 34	5 95	1 067	40 0	1 03	0 009	0 042		−2	2,500	Slight malted flavor, crystals
23a										+1	−3		Good flavor, scorched milk odor
23b										−1	−3		Good quality
23c										−1	−3		Pungent flavor
24										−1	−3		Good quality
25										+2	−3		Good quality, slight churning

SUMMARY

Feathering of evaporated milk in hot coffee is promoted by: (*a*) Characteristics of the milk, especially the salt balance; (*b*) coffee which is concentrated; (*c*) prolonged extraction of the coffee grounds; (*d*) the use of small quantities of milk in the coffee; (*e*) the slow, rather than fast, addition of milk to the coffee.

An imperical feathering test was developed.

Examination of 52 cans of commercial evaporated milk indicated that feathering in hot coffee is not a common defect of the product now offered to the public.

REFERENCES

(1) BURGWALD, L. H.: Some factors which influence the feathering of sweet cream in coffee. Jour. Agr. Res., xxvi, no. 11, (1923).

(2) DOAN, F. J.: Homogenization affects protein stability. Milk Dealer, xix, no. 3, 57, (1929).

(3) DOAN, F. J.: Relation of feathering and heat stability of cream to fat clumping due to homogenization. Paper presented before the 1930 Annual Meeting of the American Dairy Science Association, Ames, Iowa.

(4) HUNZIKER, O. F.: Condensed milk and milk powder. La Grange, Ill, (1926).

(5) ROGERS, L. A., DEYSHER, E. F., AND EVANS, F. R.: The relation of acidity to the coagulation temperature of evaporated milk. Jour. Dairy Sci., iv, 294, (1921).

(6) SOMMER, H. H., AND HART, E. B.: The heat coagulation of evaporated milk. Wisconsin Technical Bulletin No. 67, (1926).

(7) TRACY, P. H., AND RUEHE, H. A.: A case of cream feathering. Creamery and Milk Plant Monthly, xvii, No. 521, (1928).

(8) WEBB, B. H., AND HOLM, G. E.: The heat stability and feathering of sweet cream as affected by different homogenizing pressures and different temperatures of forewarming. Jour. Dairy Sci., xi, 243, (1928).

SKIMMILK AS A FEED FOR DAIRY COWS*

C. H. ECKLES AND E. N. SHULTZ

Division of Dairy Husbandry, University of Minnesota, St. Paul

In the butter-producing areas of the United States the proper utilization of the by-products is an important economic question. Minnesota, for example, with a total butter production of approximately 290,000,000 pounds annually has a total of over six billion pounds of skimmilk and buttermilk available for use on the farms and in the factories. The recommendations of agricultural authorities have been to use the dairy by-products for feeding livestock and the excellent results following this general practice are well known.

In recent years the production of cream for butter manufacture has developed in regions where it is not practical to produce sufficient grain to make swine production profitable on a large scale. The farmer uses all the skimmilk he can as feed for his pigs, calves, and chickens and still often has milk on hand. A calculation based upon census figures shows that after allowing for all the skimmilk that can be used economically by the pigs, calves, and poultry in this state a surplus of over a billion pounds remains. Most of this is not entirely wasted but is used in excessive amounts as feed for livestock. It is well established that the special efficiency of skimmilk in the diet of the growing pig is due to the excellent quality of the protein which supplements the rather low quality proteins of cereals, and to a lesser extent to the mineral and vitamins content. The value per unit of

---

* Published with approval of the Director as Paper No. 959, Journal Series, Minnesota Agricultural Experiment Station. The data used are taken largely from the thesis of E. N. Shultz submitted in partial fulfillment for the degree of Master of Science, University of Minnesota, June, 1929.

Received for publication August 27, 1930.

skimmilk in the ration rapidly declines when fed in amounts beyond that needed to supplement the grain. In this case the milk solids serve merely as a source of digestible nutrients.

Records taken by the Division of Farm Management and Agricultural Economics of the University of Minnesota from a cost accounting route including 23 farms in Pine County, Minnesota illustrate the situation where a surplus of skimmilk is available.

With a total milk production of approximately 2,000,000 pounds, 328,320 pounds of skimmilk were unaccounted for after feeding the livestock all that could be used. Twenty-three farms on the average fed pigs 1276 pounds of skimmilk for each 100 pound of pork produced. On six out of the 23 farms skimmilk was fed to cows and on three to the horses as well. On most of the farms, curd prepared as in the manufacture of cottage cheese, was fed poultry as a means of increasing the consumption. The uneconomical use of the skimmilk is shown especially by the high rate of feeding to swine. As a result of feeding trials by various investigators it appears well established that the most economical use of skimmilk or buttermilk is by feeding it at the rate of about 525 pounds to the hundred of pork produced.

The attempt to find economical uses for skimmilk is further illustrated by a report from the tester for a Herd Improvement Association in Northern Minnesota that 23 out of 26 members were feeding skimmilk to cows as the result of recommendations by the experiment station. The use of skimmilk in this manner appears to be the proper course in view of the situation which makes impractical the raising of sufficient swine to consume the by-products available and the further fact that the character of the crops grown are such that it is necessary on most farms to buy high protein concentrates to supplement the farm grown feeds.

The alternative to some such disposal is the organization of marketing units to handle whole milk and to utilize the by-products for commercial purposes. This may be the ultimate solution but until this comes about the skimmilk can be used to advantage on the farm in place of high protein concentrates.

Eight pounds of average skimmilk contains almost exactly the same amount of digestible crude protein and total digestible nutrients as one pound of linseed oilmeal. The high biological value of milk protein is well known. On these grounds eight pounds of skimmilk should equal in feeding value one pound of linseed meal. The Dairy Division of the University of Minnesota has recommended that skimmilk be fed to cows when sufficient numbers of growing pigs, calves, or poultry are not available to utilize it in the usual manner.

In making this recommendation the fact was not overlooked that some objection to the practice may be raised from the disease standpoint, more especially in connection abortion disease. The opinion of the experiment station veterinarians is, that although this question may be raised with good reason, it would not seem especially serious in view of the fact that where the disease is present in a herd the members are subject to possible infection in other ways and the feeding of milk is not for this reason particularly significant.

Although in some sections the feeding of skimmilk to cows is by no means an uncommon practice, very little experimental data are available dealing with the subject. Beach and Clark of the Storrs Experiment Station (1) reported a feeding trial with 24 cows, although only 4 of the number would drink the skimmilk. In the trial using three animals, and nine checks skimmilk was substituted for half the grain at the rate of eight pounds to one of grain. The milk production was approximately 6 per cent greater during the period when skimmilk was fed.

Arnett (2) conducted an eighty-four day trial with two groups of 4 cows each. The two groups received the same amount of hay, silage, and grain and one group 20 pounds of skimmilk daily in addition. The group receiving the skimmilk produced approximately 10 per cent more milk. In neither of these experiments was skimmilk used primarily as a protein supplement which is clearly the place it should fill in the ration for the most economical results.

Two German workers, Honcamp and Helms (3), recently reported a feeding trial in which skimmilk was compared with a high protein supplement as part of a ration for lactating cows.

Using the reversal plan, eight cows were fed for three periods of twenty days each with ten days preliminary periods. During the first and third periods each cow received 22.5 pounds of skimmilk daily. During the second period the skimmilk was replaced with soybean oilmeal and dried beet pulp on the basis of starch value and digestible protein. The production during the period of skimmilk feeding was essentially the same as for the period of soybean meal feeding. The authors conclude that although skimmilk represents a useful high protein feed, it is best adapted for the feeding of pigs and calves. The market value of the skimmilk was given as twice its feed equivalent in soybean meal and beet pulp. On account of the lower value of skimmilk in the United States this conclusion would not apply here.

Before experimental trials were made it was considered reasonably safe to base recommendations regarding the use of skimmilk as part of the ration for dairy cows upon the well established facts concerning the composition of skimmilk and the nature of the proteins. However, it seemed advisable before going very far, to confirm these conclusions by means of some experimental results. Accordingly tests were arranged to compare the results of replacing linseed oilmeal in the ration with skimmilk on the basis of 8 pounds of skimmilk to one of the meal. A trial was made with lactating cows and another with growing dairy heifers.

In the test with cows 9 animals were used following the double reversal plan in which group A receiving skimmilk in periods 1 and 3, and linseed oilmeal in the second period. Group B received the oilmeal in the first and third periods and skimmilk during the second period. The periods were 24 days in length with a six day transitional period. The grain mixture for both groups consisted of ground corn 350, ground barley 250, ground oats 200, and bran 200 pounds. The roughage was mixed hay and silage. This ration was so selected to require additional protein to meet the needs of the cows. During the skimmilk periods this necessary protein was supplied by feeding skimmilk at the rate of 2 pounds to each pound of the feed mixture used. The oilmeal was fed at the rate of one-fourth pound to each pound of the grain mixture. During the skimmilk periods 27.7

per cent of the digestible crude protein was supplied by the skimmilk. The linseed oilmeal supplied 27 per cent of the protein during the periods when this concentrate was fed.

The amount of the ration to be fed was calculated by means of the Morrison Standard and adjustments in amount were made only during the transition periods. Corn silage was fed at the rate of approximately 3 pounds daily per 100 pounds of live weight. The cows were fed twice daily and milked with a milking machine. Complete feed records were kept, weights of the cows were taken for three days at the beginning and end of each period, and composite milk samples were analyzed for fat at eight-day intervals. It was found only one of the seven cows would drink the skimmilk. After some preliminary tests the plan was adopted of weighing the grain for a particular cow into a container and

TABLE 1

Milk and fat production

Skimmilk versus linseed oilmeal as protein supplements

24-DAY PERIODS	PRODUCTION PER COW			GAIN IN WEIGHT PER COW
	Milk	Fat		
	pounds	*per cent*	*pounds*	*pounds*
Group A (3 cows):				
1. Skimmilk, 24 days	691.4	4.15	28.70	26
per day	28.8		1.19	
2. Linseed oilmeal, 24 days	650.8	4.20	27.36	−29
per day	27.1		1.14	
3. Skimmilk, 24 days	577.7	4.16	24.04	24
per day	24.1		1.01	
Group B (4 cows):				
1. Linseed oilmeal, 24 days	671.2	4.12	27.68	18
per day	27.9		1.15	
2. Skimmilk, 24 days	606.0	4.24	25.70	−12
per day	25.2		1.07	
3. Linseed oilmeal, 24 days	570.5	4.25	24.29	21
per day	23.7		1.01	

pouring the desired amount of skimmilk on the grain just previous to placing it before the cow. The grain mixture absorbed the milk and no loss of skimmilk occurred when the feed wet with the milk was placed on top of the silage.

Table 1 shows the average milk yield for group A was slightly in favor of linseed meal, for group B the skimmilk period showed a small lead. This difference is considered too small to be significant. It is recognized that the numbers of cows used was rather small but in view of the fact that the results corroborate those which would be expected from the known nutritive values of the feeds, it seems safe to accept them as indicating the value of skimmilk under the condition described. The increase in weights of the animals used was probably the result of a slight over-feeding due to the calculation of the ration being made during the transition periods. When this ration was continued without change during the twenty-four-day period the natural decline in milk production resulted in a small excess of nutrients before the end of the period. The loss in weight for both groups during the second period is accounted for by weather conditions. The experiment was not set up in such a manner as to give indications of a possible greater value for milk productions of the proteins from the skimmilk because the amount of protein was presumably sufficient in the ration received by both groups.

FEEDING TRIALS WITH HEIFERS

The value of skimmilk for feeding the calf up to the usual age of weaning is well known and is not a part of this consideration. It was the purpose to determine if 8 pounds of skimmilk would serve the same purpose in the ration as one pound of linseed meal for animals between the usual age for milk feeding and maturity.

Six heifers were used ranging in age from seven to twelve months divided into two groups. The trial covered 90 days. The roughage was timothy hay of a good grade. With this was fed a small allowance of ground corn supplemented for one group by linseed oilmeal, for the other by skimmilk. The low protein roughage was selected in order that a fairly large amount of protein would be necessary as a supplement. The results are given in table 2. The comparison in gains is made to the Eckles' normal figures (4).

It will be noted that two calves in each lot greatly exceeded the normal gain while one in each lot fell behind. The average gain of 1.11 pounds per day for the group receiving skimmilk is almost identical with the figure 1.14 from the group fed linseed oilmeal. As in the case of the cows in milk 8 pounds of skimmilk appear to be equal to one pound of linseed oilmeal as a supplement to ordinary rations lacking in protein. It is probable that under certain conditions the higher biological value of milk protein would widen this ratio. The calves receiving the skimmilk supplement made distinctly greater skeletal growth than the group receiving the oilmeal, but the numbers used were too small to justify any conclusions.

TABLE 2

Skimmilk versus linseed oilmeal as a supplementary feed for dairy heifers

CALF NUMBER	AGE BEGINNING	WEIGHT BEGINNING	TIMOTHY HAY PER DAY	CORN PER DAY	LINSEED OILMEAL	SKIM MILK PER DAY	GAIN PER DAY	PER CENT NORMAL GAIN
	days	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*per cent*
1	332	616	13 6	2 25		18	1 31	177
2	236	523	9 2	2 0		16	1 24	105
3	247	347	6 8	1 8		14	0 79	78
Average							1.11	
4	331	645	13 6	2 25	2 25		1 40	189
5	338	371	8 0	2 0	2 0		1 06	138
6	213	393	7 8	2 0	2 0		0 95	77
Average							1 14	

DISCUSSION

The number of animals used in the trials reported was limited but it is felt the results may be accepted in view of the fact that these experimental data confirm the results expected from a comparison of the nutritive values of skimmilk and linseed oilmeal. It is probable with a more careful set up of experimental conditions a higher nutritive quality of milk protein for milk production could be observed. The more rapid skeletal growth of the heifers receiving the skimmilk may be an indication of this factor. In ordinary feeding practice the possibility of better results from the skimmilk due to the quality of the protein could be ignored on account of the limited information available and the difficulties of applying the present knowledge to the complicated situation resulting from the use of mixed rations.

It is clear that the value of skimmilk as a feed for cows will depend largely upon the character of the ration with which it is fed. If fed, as has been done by some farmers, by merely adding to a ration already meeting the requirements of the animals reasonably well the skimmilk serves only as a source of additional calories and protein. Under these conditions of feeding the fairest comparison probably is on the basis of the total digestible nutrients. On this basis with corn at $30 per ton skimmilk would be worth 16 cents per 100 pounds. On a protein basis, if linseed oilmeal costs $55 per ton skimmilk should be worth 33 cents per hundred. To use skimmilk economically as a feed for cattle beyond the usual age for milk feeding requires that the ration be so formulated as to depend upon the skimmilk for a considerable proportion of the protein. It is clear in regions where the feed grown on the farms contain an abundance of protein, or low protein feeds are not cheaper on the market, there is small reason for considering the feeeing of skimmilk to mature animals. The place where it may be done with advantage is in the middle west where a surplus of skimmilk is available on many farms and where the crops grown are such as to require the purchase of high protein concentrates as supplementary feeds.

It is possible the present surplus of skimmilk will disappear in time with a reorganization of the dairy manufacturing units which will result in facilities for using the by-products to better advantage. At present only about 5 per cent of the skimmilk and buttermilk available in Minnesota is used for commerical purposes and the price paid the producer for the product is no more, and is often less, than he can realize by feeding the skimmilk to his farm animals under proper conditions. The use of skimmilk as a feed for cows and growing heifers extends the possibilities of using the surplus skimmilk to advantage on the farm.

CONCLUSIONS

1. Skimmilk can be used as a protein supplement in the ration for dairy cattle, replacing linseed oilmeal when fed at the rate of 8 pounds of skimmilk to 1 pound of linseed oilmeal.
2. The feeding of skimmilk as a source of protein to dairy cattle is an economical practice in areas where the use of surplus skimmilk is a problem.
3. The value of skimmilk for feeding purposes is determined by the market prices of the high protein concentrates which can be purchased.
4. Skimmilk can be used economically to supply protein in the rations of young dairy stock fed a low protein roughage such as timothy hay.

REFERENCES

(1) Beach, C. L., and Clark, A. B.: Feeding skimmilk to dairy cows. Storrs Exp. Sta Report 1904, 148–149.
(2) Arnett, C. N.: Skimmilk for dairy cows. Mont. Exp. Sta. Report 1921, 34–36.
(3) Honcamp, F., and Helms, W.: Die verwertung der Magermilch als eiweissreiches Beifutter durch die Milchkuh. Milchwirtschaftliche Forschungen., 7: 502–509 (1929).
(4) Eckles, C. H.: The normal growth of dairy cattle. Mo. Exp. Sta. Research Bul. 36: 1–20 (1918).

THE EFFECT OF ACIDITY ON LACTOBACILLUS ACIDOPHILUS CULTURES*

LUTHER A. BLACK† AND J. C. HARRIS

Division of Dairy Husbandry, Agricultural Experiment Station and Department of Bacteriology, State College of Washington, Pullman

Some effects of storage on *Lactobacillus acidophilus* cultures have already been reported (1). It was concluded that *L. acidophilus* milk cultures could be stored at either refrigerator or room temperatures for several days without any considerable destruction of the organisms, provided that the original acidity was not too high. The results then obtained were secured with milk cultures of original acidities from 0.50 to 0.80 per cent, expressed as lactic acid.

The data here presented were obtained upon milk cultures of *L. acidophilus* that were high in acidity when stored.

PROCEDURE

In addition to strain A4 and A15 previously reported upon for low acidities,[1] three additional strains were used, A2, A5 and A10. These strains were all stock cultures.

Flasks containing 100 cc. of skimmed milk were sterilized by heating at 15 pounds pressure for twenty-five minutes. After cooling, they were inoculated with 1 per cent of a milk culture of the respective strains and incubated at 37°C. A fresh flask for each strain was inoculated at twelve-hour intervals for a period of eighty-four hours. This resulted in a total of 8 flasks for each strain, each having a twelve-hour-longer incubation period than the next one in the series.

---

* Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 174, College of Agriculture and Experiment Station, State College of Washington. Received for publication September 5, 1930.

† Present address, University of Maryland, College Park, Md.

[1] Black, L. A. 1931 Viability of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* cultures stored at various temperatures. Jour. Dairy Sci., xiv, 59–72.

The flasks were allowed to remain in the incubator until after the most recently inoculated flask had coagulated. Since the rate of coagulation varied somewhat among the strains, this resulted in a longer period of incubation in some cases. All flasks of each strain were removed from the incubator at the same time, thus giving cultures of varying ages. This also resulted in corresponding variations in acidity.

The acidity of each culture was determined by titration of a 10-cc. sample. A sample was removed for plating after which each culture was divided, one portion was left at room temperature and the other was placed in the refrigerator. The room temperature varied from 20° to 24°C. and the refrigerator temperature was approximately 9°C. Three cultures were left at these temperatures for five days, two for six days and 1 culture for nine days, when the acidity was again determined and plates were poured.

Bacto-dehydrated whey agar was used for all plating. Duplicate plates were poured and incubated at 37°C. for four or five days. All strains were incubated in an atmosphere containing 5 per cent carbon dioxide, except the first experiment on strain 4 which was in an atmosphere containing 10 per cent carbon dioxide. These percentages of carbon dioxide had been found by previous experiment to favor the growth of the strains used. Water in a flask was displaced by carbon dioxide from a tank, and then by replacing measured amounts of water in the flask, the carbon dioxide was forced into the petri plate containers which, in these experiments, were vertical autoclaves. In this way carbon dioxide, to the extent of 5 or 10 per cent of the volume of the container could readily be measured.

No circulation of the carbon dioxide was attempted with strains 4, 2 and 5. During the incubation of the strains 4a, 10 and 15, the atmosphere surrounding the plates was kept in slow circulation. This was accomplished by pumping the air from the bottom of the container and returning it at the top of the petri plate container, using a closed circulation system.

RESULTS

The results obtained are presented in table 1.

TABLE 1

Viability of L. acidophilus cultures at various acidities

CULTURE	INCUBATED	ORIGINAL		STORED	REFRIGERATOR		ROOM	
		Acidity	Count		Acidity	Count	Acidity	Count
	days	*per cent*	*millions*	*days*	*per cent*	*millions*	*per cent*	*millions*
4-1	5	1.65	56.0*	9	1.82	—	1.91	—
4-2	4.5	1.58	192.5		1.49	31.0	1.86	—
4-3	4	1.52	103.0		1.70	—	1.92	—
4-4	3.5	1.40	187.0*		1.47	63.5	1.76	—
4-5	3	1.14	186.0*		1.35	256.5	1.67	107.0*
4-6	2.5	0.87	92.0*		1.20	—	—	—
4-7	2	0.73	106.0		1.04	282.0*	1.33	196.0
4-8	1.5	0.44	89.0		—	—	—	—
2-1	7	1.88	33.5	6	2.12	—	2.16	—
2-2	6.5	1.79	63.0		2.14	—	2.11	—
2-3	6	1.72	117.0*		1.91	7.9	2.08	20.5*
2-4	5.5	1.36	40.0		1.93	—	2.09	—
2-5	5	1.40	97.0		1.68	24.1	1.93	20.1
2-6	4.5	1.23	42.5		1.53	81.0	1.79	61.0*
2-7	4	0.89	189.0		1.13	—	—	—
2-8	3.5	0.55	82.5		0.91	67.0	1.32	39.5
5-1	7	1.62	6.0	6	1.86	—	2.34	—
5-2	6.5	1.58	35.0		1.85	—	1.83	—
5-3	6	1.50	87.0		1.69	19.6	1.69	—
5-4	5.5	1.35	113.5		1.68	31.9	1.71	22.1
5-5	5	1.31	93.0		1.49	30.5	1.62	—
5-6	4.5	1.22	81.0		1.39	52.7	1.58	10.1*
5-7	4	0.95	219.0*		1.31	120.0	1.36	—
5-8	3.5	0.71	221.0		0.99	194.0	1.07	86.0
4-a1	5	1.89	36.0	5	1.78	118.5	1.92	3.0
4-a2	4.5	1.75	126.5		1.76	88.0	1.78	10.0
4-a3	4	1.73	352.5		1.66	130.0*	1.74	87.5
4-a4	3.5	1.59	173.0		—	313.5	1.64	130.0
4-a5	3	1.55	319.5		1.45	265.0	1.62	165.0
4-a6	2.5	1.15	361.5		1.39	186.0*	1.49	173.0
4-a7	2	1.00	542.0*		1.39	410.0	1.49	216.0
4-a8	1.5	0.92	144.0*		1.15	363.0	1.27	197.0

* Result of one plate.

— No count, usually moulds.

TABLE 1—*Concluded*

CULTURE	INCUBATED	ORIGINAL		STORED	REFRIGERATOR		ROOM	
		Acidity	Count		Acidity	Count	Acidity	Count
	days	*per cent*	*millions*	*days*	*per cent*	*millions*	*per cent*	*millions*
10–1	4.5	1.67	11.2	5	1.67	6.3	1.73	6.7
10–2	4	1.62	15.7		1.61	11.5	1.72	7.4
10–3	3.5	1.52	1.3		1.46	22.5	1.66	21.1
10–4	3	1.35	51.0		1.26	3.5	1.64	11.4
10–5	2.5	1.30	44.5		1.17	3.8	1.27	20.5
10–6	2	1.19	392.5		0.96	13.3	1.04	20.5
10–7	1.5	0.68	531.5		0.79	15.5	0.87	26.6
10–8	1	0.45	302.0		0.55	16.0	0.74	23.9
15–1	5	1.87	2010.0*	5	1.89	2250.0	1.90	1950.0
15–2	4.5	1.88	1750.0		1.82	2110.0	1.93	2130.0
15–3	4	1.63	2200.0		1.80	2800.0	1.89	2350.0*
15–4	3.5	1.64	2260.0		1.71	1960.0*	1.77	2190.0*
15–5	3	1.43	2330.0		1.38	2340.0	1.78	3530.0
15–6	2.5	1.24	2735.0		1.28	2515.0	1.47	2465.0
15–7	2	0.93	2090.0		1.07	1715.0	1.31	2500.0
15–8	1.5	0.85	1900.0		1.09	780.0	1.24	2160.0

DISCUSSION

The results presented in table 1 indicate that the highest count of organisms was obtained at the lower or moderate acidities and that the greatest destruction of organisms occurred in the more acid cultures. The original cultures will be considered first in the discussion to follow; that is those cultures that were not stored. They will be discussed in the order given in table 1.

It is believed that the factor of age can be largely eliminated in the consideration of the results presented in table 1. In culture 10, however, it seems that age or some other factor operated in drastically reducing the number of organisms in all samples upon storage. It is not likely that acidity could here be the predominating factor since at the low acidity of 0.55 per cent the same severe reduction was obtained as occurred at the higher acidities. This factor might also account for the more rapid reduction occurring with this culture, strain 10, in the higher acidities of the original series.

The highest counts in strain 4 were obtained at acidities of 1.14, 1.40 and 1.58 per cent. Samples were not obtained between 0.87 and 1.14 per cent acidity. Judging from the refrigerator storage samples of this series, and from the second set of results upon this strain, given in table 1, it seems probable that the greatest number of organisms was reached between the acidities of 0.87 and 1.14 per cent. Destruction of the organisms in strain 4 had probably started at 1.14 per cent acidity. The number of viable organisms then remained rather constant through 1.58 per cent with a greater decrease at 1.65 per cent acidity.

The highest count in strain 2 was obtained at an acidity of 0.89 per cent. The next highest acidity in the series was 1.23 per cent. It seems likely that the greatest number was reached between these acidities. This would then correspond closely with the results obtained with strain 4. Destruction of the organisms had probably occurred at 1.23 per cent acidity and the number surviving then remained more or less constant through the remainder of the series.

The highest counts in strain 5 were obtained at acidities of 0.71 and 0.95 per cent. This is somewhat lower than in the case of the strains already mentioned. Destruction of the organisms had started at 1.22 per cent acidity. The number of organisms remained about the same through 1.5 per cent but increased destruction took place at 1.58 and still more at 1.62 per cent acidity.

In the second series of experiments on strain 4,. the largest number of organisms were obtained at 1.00 per cent acidity. This corresponds to the conclusion reached in the first series as judged by the refrigerator storage samples. Destruction in the second series had begun at 1.15 per cent acidity. The number of organisms remained somewhat the same through acidities to 1.73, decreasing at 1.75 and decreasing greatly at 1.89 per cent acidity.

The largest number of organisms in the series of strain 10, were obtained at 0.68 per cent acidity. This resembles strain 5, which had the highest count at an acidity lower than strains 2 and 4. Destruction of organisms in strain 10 had commenced at 1.19 per cent acidity. A great destruction had again occurred at 1.30

per cent acidity, the number of organisms then remaining more or less constant until further decreases took place at acidities of 1.5 per cent and above. As mentioned above, some of this destruction at 1.3 per cent acidity may be due to the greater effect of age or other factors upon this strain.

Strain 15 has always differed from some dozen other strains of *L. acidophilus* that have been examined. This strain was used because it was received from a laboratory specializing in acidophilus cultures, and was stated to be recently isolated from the human intestinal tract. For about two years this culture has given some ten times as high a count as other acidophilus strains that have been used.

Strain 15 also seems to be more resistant to acid than the other strains used. An earlier experiment on this strain, similar to the series reported in table 1, gave essentially the same results. This earlier series could not be counted inasmuch as a one million dilution was the highest used. At the end of the experiment, however, the plates were all placed in order and a rough comparison made. Destruction of organisms could not be detected in the lower acidities until an acidity of approximately 1.5 per cent was reached. At the higher acidities somewhat greater reductions occurred than in the present series of experiments. It can be concluded, therefore, that strain 15 is more resistant to the acid formed than are the other cultures used in the present experiments.

The largest number of organisms in strain 15 was secured at 1.24 per cent acidity in the original set of cultures. This is at a higher acidity than was found for strains 2 and 4 and considerably higher than strains 5 and 10. That it is really higher is borne out by the highest counts in the stored samples, which were obtained at still higher acidities. Destruction of the organisms in the original set had started at 1.43 per cent acidity. Further decreases in numbers were indicated after 1.88 and 1.87 per cent acidity.

The results indicate then, that the highest number of organisms reached with regard to acidity was as follows: strain 4, 0.87 to 1.14; strain 2, 0.89 to 1.23; strain 5, 0.71 to 0.95; strain 4, 1.00;

strain 10, 0.68, and strain 15, 1.24 per cent. Destruction had apparently occurred at the following acidities: strain 4, 1.14; strain 2, 1.23; strain 5, 1.22; strain 4, 1.15; strain 10, 1.19, and strain 15, 1.43 per cent. A still more pronounced destruction of organisms had taken place in each of the strains except 2 at the following acidities: strain 4, 1.65; strain 5, 1.58 and 1.62; strain 4, 1.75 and 1.89; strain 10, 1.3 and 1.52, and strain 15, 1.87 per cent.

In the data presented strains 2 and 4 gave the highest plate counts at approximately 1.00 per cent acidity, strains 5 and 10 at less than 1.00 per cent and strain 15 at an acidity above 1.00 per cent. A decrease in the number of organisms seemed to have occurred when 1.2 per cent acidity had been reached in all strains except 15. There was considerable variation in the higher acidities at which still greater decreases in viable organisms were evident.

When we consider the highest numbers secured and the acidities at which a decrease occurs in the storage samples, we find that they correspond to the original unstored samples. The highest number of organisms, again, are found at the low acidities and the greatest destruction of organisms takes place in the more acid of the series.

In the cultures stored in the refrigerator the highest number in strain 4 was secured at 1.04 per cent acidity with a decrease at 1.35 per cent. With strain 2 counts were not secured between acidities of 0.91 to 1.53 per cent. It seems probable that destruction had begun at 1.53 per cent acidity or before with the highest number below this. In strain 5 the highest number was secured at 0.99 and decrease had taken place at 1.31 per cent acidity.

Continuing with the other refrigerated cultures, the second series of strain 4 exhibited the largest count at 1.39 per cent acidity. However, in another culture of the same acidity the number of organisms had already decreased. So it is probable that a decrease had already occurred at 1.39 per cent acidity and that the highest number had been reached before this.

As mentioned in the second paragraph of this discussion, some other factor seems to have operated in greatly reducing all storage samples of strain 10. Strain 15, as mentioned above, seemed to be more resistant to the acidity produced than other strains. So in the refrigerated samples, a large number of organisms was secured at 1.28 per cent, followed by a decrease at 1.38 and 1.71 per cent. In this series the highest number was secured at an acidity of 1.80 per cent, followed by a decrease at 1.82 and 1.89 per cent acidity.

In the samples stored at room temperature, of strains 2, 4, 5 and the second series of strain 4, the highest count was in all cases secured in the sample corresponding to that giving the highest count in the refrigerated samples. In these cases there had been an increase in acidity of from 0.08 to 0.29 per cent in the samples stored at room temperature over that of the refrigerated samples.

In every case the highest count for the room storage samples was less than the count of the corresponding refrigerated sample. Decrease in numbers among a series of these room stored cultures proceeded like that of the refrigerated samples, except that in every instance the acidity for the corresponding culture was higher at room storage and in all but one case, culture 2–3, the count was correspondingly lower.

The general effect of storage in the refrigerator of the cultures at the various acidities was to decrease the numbers. In 11 of 38 samples, however, there was an increase in count above that of the original. Of these increases, 3 were at lower acidities, suggesting multiplication before chilling. In 4 other cases, it seems from the data that the counts were higher largely because the original count for that series was lower than would be expected from the other younger and older cultures of its series. The remaining 4 instances of an increased count were in strain 15.

The general effect of storage at the various acidities, then, is to decrease the numbers upon storage at 9°C. with a further reduction in numbers if stored at 24°C. In the first four strains presented in table 1, in only one case, culture 2-3, was there an increased number of organisms in room storage over refrigerator storage. In strain 10 the opposite effect prevails, but other factors must be involved there. With strain 15, in 5 instances a higher count was secured at room storage than with refrigerator storage. In 15-8 and 15-7 it would seem that continued multiplication accounted for this increase at room storage. Whether this could explain the other three increases of the series is problematical.

The acidity in all cases but one was higher at room storage than at refrigerator storage. In this one instance the difference was 0.03 per cent. In all but 10 of the 48 refrigerated samples, the acidity had increased over that of the original. In these 10 instances, the variation was from 0.01 to 0.23 per cent, all but one being 0.11 or under, all averaging 0.095 per cent with 6 of the 10 samples below this average. It is probable that much of this difference might be due to difficulty in reaching the same end point on each titration, since the sterilized milk is slightly caramelized and so interferes with the color obtained at the endpoint.

In all but 2 of the 48 room storage samples, the acidity had increased over that of the original. These cases, 10-6 and 10-5, were 0.15 and 0.03 per cent lower and were the two samples that varied the most in the refrigerated samples. This might presumably have been due to too high a reading on the original set.

None of these cases of variations in acidity, which might have been due to error in recording the endpoint of the titration, concerned a series at which the highest number of organisms was secured. In only one case, 10-6, this might have resulted in a different acidity at which decrease was first evidenced. In the other instances of acidity variations the samples concerned were above that acidity in which decrease was first noticeable.

In connection with the experiments presented in table 1, the long period of incubation of *L. acidophilus* plates also pointed out conditions not noticeable in ordinary plate counts of shorter incubation periods. Molds did not appear within the two-day incubation period for standard plate counts, but with a five-day incubation of acidophilus organisms, plates sometimes could not be counted because of mold growth. These molds usually appeared as one or two large colonies covering most of the plate. The molds seemed to enter the plates after the plates were inverted and placed in the incubator. After growing down between the inverted outer and inner dishes and then up inside the inner plate to the media, the mold spread from the edge of the plate, covering large areas. In certain experiments in closed containers, it seemed that a very few mold spores could thus grow and spread from one plate to another, infecting practically every plate within five days incubation.

From the results given, it seems that the method of incubating until different acidities are reached, plating at these acidities and drawing conclusions from the counts obtained, gives evidence regarding the effect of storage at the various acidities. The largest counts in the original set of samples were obtained at acidities lower than was the case with the stored samples, yet in general the samples giving highest counts upon storage was identical with those giving the highest count in the original. This would indicate that the increase in acidity before chilling was completed was not particularly harmful if the cultures were removed from the incubator when the maximum number of organisms was reached. If the cultures were to be stored at room temperature, it is reasonable to suppose that at some acidity under that giving the maximum number in the original there would be slightly more multiplication at room temperature and so yield more organisms after storage than would result from storage of the sample giving the maximum number before storage.

It is suggested that results might be more readily obtained by using different amounts of inoculation and plating more than once from the same sample. This should enable one to secure samples within a closer range of acidities than was possible in the experiments here presented.

SUMMARY

1. The strains of *L. acidophilus* used in the experiments reported upon gave the highest plate counts at acidities of approximately 1.00 per cent.
2. There was some variation among strains, some yielding the maximum number of organisms at less than 1.00 per cent and one strain at an acidity considerably greater than 1.00 per cent.
3. A decrease in the number of organisms had taken place at an acidity of approximately 1.2 per cent in all but one strain.

The strains examined seemed to differ slightly with respect to the acidity at which destruction became apparent.

4. At higher acidities of approximately 1.5, 1.6, 1.7 and 1.8 per cent, a still greater destruction of organisms took place. The acidity at which this greater destruction occurred varied with the strain.

5. The results indicated that the particular sample of each strain that gave the highest count before storage, usually gave the highest count of the series after storage at 9° or 24°C.

6. Refrigerator storage (9°C.) of typical strains of *L. acidophilus* resulted in a smaller loss in the numbers of organisms than did storage at 20° to 24°C., in both high and low acidities.

7. Two strains of *L. acidophilus* not previously reported upon also survived either refrigerator or room temperature storage for several days without any great destruction of organisms, provided that the original acidity was low.

8. It is desirable to determine the acidity at which the maximum number of organisms is secured for each strain and under the conditions that obtain.

9. The results obtained indicated that the acidity at which the maximum number of organisms was secured during incubation of a culture was an index as to the acidity at which to place cultures in refrigerator storage.

THE NUMBER OF DAUGHTERS NECESSARY TO PROVE A SIRE*

JAY L. LUSH
Iowa State College, Ames, Iowa

Increased appreciation of the progeny test as a means of rating the real breeding worth of an animal is a noteworthy development in modern animal breeding. The idea of a progeny test is not a new one. The best breeders have always relied upon it, although in a somewhat indefinite and approximate way. Why a progeny test is more accurate and useful than a rating of the individual by what it appears to be itself, or by what its pedigree shows that its ancestors were, began to be evident soon after the rediscovery of Mendelism but only in the last ten years have adequate methods been available for combining genetics and statistical procedure to study the usefulness of the progeny test under varying conditions.

It has always been known in a general way that a progeny test increases in reliability as the number of offspring included in that progeny increases. That this increase in reliability might be expressed very definitely by a fairly simple formula was first suggested by critical examination of Illinois Station Bulletin 270, which appeared in 1925. This bulletin was an empirical study of bulls having 15 or more daughters entered in the Register of Merit of the American Jersey Cattle Club. Figure 1 is modelled after figure A of that bulletin with three smooth lines showing the theoretical results if the simple correlation between the records of half sisters had been 0.25, 0.30, or 0.36 and if the number of bulls tested had been large enough to equalize all errors of random sampling.

The study in that bulletin was limited to the correlation between a small number of daughters and some arbitrarily chosen larger number. It is possible to go a step further and derive a

---

* Received for publication September 12, 1930.

general formula[1] for the correlation between the average record of the daughters and the genotype of the sire for factors affecting milk production.

In deriving these general formulas allowance should be made for the fact that the primary correlation between the records of half sisters is in itself a compound figure, made up partly of the fact that half sisters tend to have received somewhat similar inheritance from their sire, and partly of the fact that half sisters are usually reared in the same herd and are therefore to some extent subject to common conditions of environment, feeding and system of management. Also the dams of the half sisters may in general have an appreciable relationship to each other. This compound nature of the simple correlation between the records of half sisters is shown in figure 2, which is drawn according to Dr. Wright's path coefficient method of analyzing correlation coefficients into their component parts. If we let s represent the path coefficient from the sire's genotype to the daughter's record and if we let e represent the path coefficient from the herd management or common environment (including also the average relationship of the dams to each other) to the daughter's record then it is evident that the primary observed correlation between the records of half sisters equals $e^2 + s^2$. (This formula does not allow for relationship between sire and dam, but such relationship is negligible in most material on account of the widespread avoidance of all systems of mating animals very closely related to each other.) Besides the number of daughters included in the average, the formulas in figure 2 require an evaluation of *s* and of *e* before they can be solved in numerical terms. The value of *s* would be 0.50 in a random-bred population where there was no dominance and where milk and fat production were entirely determined by heredity. If dominance is at all widespread in the factors affecting milk and fat production this value would be slightly lowered. We know that milk and fat production are not entirely determined by heredity and this also would lower the value of *s*. On the

---

[1] The mathematical and genetic principles underlying such formulas as this have been stated by Dr. Sewall Wright ("Correlation and Causation," Jour. Agr. Research, xx, 557–585, and "The Biometric Relations Between Parent and Offspring," Genetics, vi, 111–123). A correlation coefficient measures the tendency of two things to vary together and thus to be predictable from each other. In this case the correlation between the genotype of the sire and the average record of his daughters measures how closely the real breeding worth of the sire for milk and fat production can be predicted from the records of the daughters. A correlation of 1.0 would mean that the bull's breeding worth is perfectly predictable, with no room at all for error. A correlation of 0 9 would mean that predicted values and actual values will be nearly alike and most of the errors which do occur will be small ones. In general a correlation of 0.5 expresses the resemblance between one parent and one offspring while a correlation of 0.7 expresses the general tendency of one offspring to be like the average of both its parents where (1) neither the parents nor the offspring are highly inbred or distinctly crossbred, (2) the character being studied is not markedly influenced by the variations in environment to which that population is exposed, and (3) where few if any of the genetic factors, governing the characteristic being studied, are completely dominant or complementary in their action.

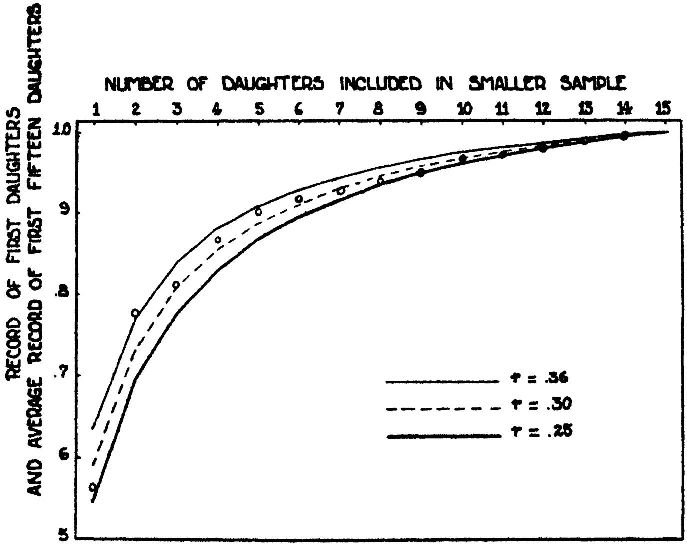
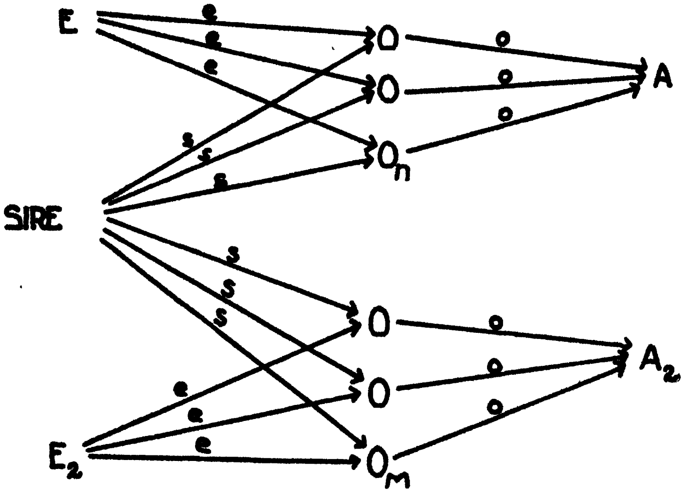


FIG. 1. THEORETICAL AND OBSERVED CORRELATIONS BETWEEN THE AVERAGE RECORD OF THE FIRST FEW DAUGHTERS AND THE AVERAGE RECORD OF THE FIRST FIFTEEN DAUGHTERS

The circles show the values actually found when the original data on which Illinois Bulletin No. 270 was based were recalculated, grouped in smaller class intervals than used in that bulletin. Dr. Davidson's kindness in supplying the original data made possible a more adequate test of the general formula theoretically derived.

other hand whatever has been accomplished through selective breeding of like to like among the ancestors of the sire would tend



$$R^2_{A\cdot Sire} = \frac{ns^2}{1+(n-1)(e^2+s^2)}$$

$$R^2_{A\cdot A_2} = \frac{nms^4}{[1+(n-1)(e^2+s^2)][1+(m-1)(e^2+s^2)]}$$

Fig. 2. Diagram Showing the Compound Nature of the Correlation Between a Sire's Genotype and the Average Record (A) of His Daughters

The even more compound nature of the correlation between the average record (A) of his daughters in one herd and the average record (A_2) of his daughters in another herd is also shown. O represents the record of one daughter and E represents the herd management and environment which affect all the cows in one herd but may be very different (E_2) in other herds.

to raise the value of s. It does not seem possible at present to evaluate s exactly. Its most probable value seems, taking everything into account, to be not far from 0.40 or perhaps a little above that in studies restricted to official records. In table 1, *s* has been figured at four different values; namely, 0.30, 0.40, 0.50, and 0.60, although it is doubtful that either of the extreme values is often reached in actual practice. The value of *e* is even less definitely determined but would be larger in such a group as D. H. I. A. records than it would in the advanced registry records since the cows on advanced registry tests are more nearly given every chance to show their producing ability than are cows under D. H. I. A. conditions. On the basis of all analyses yet made of advanced registry records it seems likely that the most probable value of e^2 in advanced registry data is not far from 0.15 and that it can rarely if ever be as large as 0.30. In the calculations, values of 0.00, 0.10, 0.20, 0.30, and 0.40 have been assumed for e^2. Dr. Wright in an unpublished analysis finds that the results of

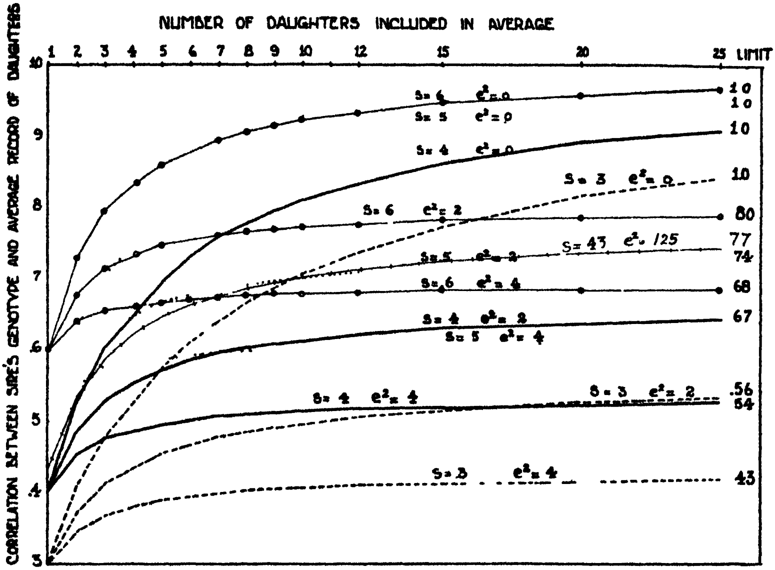


FIG 3 THE RELATION BETWEEN THE NUMBER OF DAUGHTERS IN THE PROGENY AND THE RELIABILITY OF THE PROGENY TEST

Expected correlation between sire's genotype and the average record of various numbers of daughters at selected values of *s* and e^2

Gowen's analysis of Holstein-Friesian Advanced Registry records are consistent with values of about 0.435 for s, 0.125 for e^2 (not including the relationship of the dams to each other), and 0.20 for the average relationship of the dams to each other.

Figure 3 shows the expected correlation between the genotype of the sire and the average of his daughters' records for some of these selected values of e^2 and s where the number of daughters varies from 1 to 10 and upward. These curves differ greatly among themselves but they have certain characteristics in common, most noteworthy of which is the fact that all of them rise abruptly as the number of daughters increases but that the curves tend very soon to flatten out.

Another noteworthy point about these curves is that all of them have limiting values less than 1.0 except where e^2 is 0 and even then the value, 1.0, is only approached as a limit when the number of daughters becomes infinitely large. In practical language this means that we cannot expect the progeny test to be absolutely and completely accurate even though the number of daughters is very large and every precaution is taken to control and standardize the conditions under which the records are made. When the number of daughters is small, s is in general more important than e in determining the reliability of the progeny test. If differences in herd environment and management are great in the herd or section of the breed under consideration and if the records are not corrected accurately for those differences, then the ultimate limit of the progeny test is low and for most practical purposes it increases in reliability only a little after the first half dozen or so daughters have made records. On the other hand if the effects of differences in common environment are relatively slight (or if accurate corrections are made for those differences) and if the variations in records are largely due to genetic differences between the heifers and to uncontrollable environmental forces operating differently, even on heifers in the same herd, then the reliability of the progeny test continues to increase noticeably even beyond the point where one has as many as ten or a dozen daughters already tested.

The more carefully environmental conditions are controlled and made uniform for all of the daughters which are to be tested, just so much more is the value of e lowered in making comparisons with other bulls whose daughters have been tested under similarly controlled conditions. The use of accurate correction factors for known and measured differences in herd environment serves the same purpose as actual control of the physical and biological environment, but of course can not entirely eliminate e because correction factors are only approximate, even when most carefully derived. The value of e is also slightly raised by making environmental conditions uniform throughout the herds or section of the breed under consideration (or by making proper correction for such conditions when they cannot be made uniform), since a larger proportion of the observed differences thus are determined by heredity than if conditions were less well controlled. Thus careful control of environmental conditions throughout the herd or portion of the breed considered operates to make the progeny test more reliable. This difference in reliability can become very great as may be seen by comparing the extreme outside curves in figure 3.

Such careful control of (or allowance for) environmental conditions can not entirely eliminate e^2 but may hold it down to a low level, perhaps to less than 0.10. The value of s can go above 0.5 only where a very unusual amount of inbreeding (homozygosity) is involved. Even for s to have a value of 0.6 the sire would need to be 44 per cent more inbred (homozygous) than his daughters out of unrelated or slightly related cows, or if not inbred himself he must be mated to cows more closely related to him than half sisters. That makes no allowance for the part played by dominance or by uncontrolled environmental influences in determining the daughters' records. Such a part, even though small, would necessitate still more intense inbreeding if the value of s is to approach 0.6. From these considerations it is evident that a value for s as high as 0.6 is almost unattainable with the systems of breeding prevalent in dairy cattle today. The curve for $s = 0.5$, $e^2 = 0.0$ in figure 3 probably comes close to the maximum reliability of the progeny test in any very large group of dairy herds today, even with environmental conditions as perfectly controlled or corrected for as is possible. Even if the value of s did go a little above 0.5 in certain special groups, yet e^2 would still have at least a slight value. This would result in a curve starting a little higher and rising a little steeper but flattening out sooner to a lower limit than the curve shown for $s = 0.5$, $e^2 = 0.0$.

The discussion this far has been on the assumption that the daughters' records would be used without taking into account the records of the dams of those daughters. Where the dams also have records, it seems reasonable to suppose that accuracy would be much increased by taking those records also into account. Actually this gain, though real, seems to be comparatively small. Thus, under the conditions of random mating, complete determination by heredity, and no dominance, the value of s rises from 0.50 where the dam's record is not considered at all only to 0.58 where the dam's record is compared with the daughter's record, each being weighted in the most ideal manner, i.e., with the daughter's record given twice as much weight as her dam's record. In so far as the daughters and dams are tested in the same herds the same year, the use of daughter-dam comparisons will also tend to give e^2 a lower value than if no use were made of the dams' records. From these theoretical considerations it seems that the use of daughter-dam comparisons should render the progeny test more reliable but that this effect will be less than is generally believed. It is hardly possible that s could thus be raised to the extreme value (0.6) shown in figure 3 but it might conceivably be raised above 0.5 where all environmental circumstances were controlled (or corrected for) in the most exact manner possible. e^2 could not be eliminated entirely but might possibly be held below 0.2 or perhaps even below 0.1 with excellent control (physical or statistical).

The comparison between pedigree promise and progeny test is especially interesting. For characters completely determined by heredity without dominance and in a random-bred population, the correlation between the individual and the most perfect system of averaging its ancestors is limited to about 0.71. Under the same conditions the correlation between the individual and the average of its offspring becomes this high when there are as many as three offspring included in the average. Under these same conditions when more than three individuals are available the progeny test becomes more reliable than pedigree promise. No one seems yet to have worked out the consequences of variations in *s* and *e* on the general reliability of the pedigree as an indication of what the individual will be. It is obvious that *e* will usually be smaller in a pedigree than in a progeny test since rarely will all of the ancestors have been reared under environmental conditions which were all good or all poor. On the other hand in dairy cattle there is no direct measure of productiveness in the males and this limits our ability to estimate the productiveness of an individual by a study of its pedigree. It seems likely therefore that the correlation between an animal's productiveness and what one might estimate that productiveness to be from a careful study of the pedigree, even when all female ancestors had been tested, would probably very rarely go over 0.65 and perhaps might not even be as high as 0.55 under ordinary conditions.

How then does the progeny test compare with pedigree promise as a basis for estimating an individual's real genotype for milk and fat production? Obviously there is no single answer which will fit all conditions alike. In general when there are as many as four to six offspring included in the progeny test, that test will be about as accurate as an estimate based on even a very complete pedigree. A progeny test based upon larger numbers than this will often be distinctly more reliable than all the information to be gained by careful and long-continued study of the pedigree. However there are sure to arise occasions when sires with good genotypes are used in herds where their daughters are given poor care while other bulls will be used in herds where the daughters are given excellent care and management. Under those circumstances the progeny test by itself may not be as reliable a basis for estimating that bull's heredity for milk and fat production as his pedigree would have been. On the other hand where all environmental conditions are made as uniform as possible for all of the daughters of the different bulls which are being tested, the progeny test can approach perfection as an indicator of the bull's breeding worth, while pedigree estimates must necessarily be

TABLE 1

Correlation between sire's genotype and the average record of his daughters

s	e^2	NUMBER OF DAUGHTERS INCLUDED IN AVERAGE																
		1	2	3	4	5	6	7	8	9	10	12	15	20	25	50	100	∞
0 60	0.0	0 60	0.728	0.792	0 832	0 859	0 878	0 893	0 905	0 914	0.921	0 933	0 946	0 958	0 966	0 983	0.991	1 000
	0.1	0 60	0 702	0.750	0.778	0 796	0 809	0 819	0 826	0 832	0 837	0 844	0 852	0 860	0 865	0 874	0 880	0 885
	0.2	0 60	0.679	0.714	0 733	0 745	0 754	0 760	0.765	0 769	0 772	0 777	0 782	0 786	0 789	0 796	0 799	0.802
	0 3	0.60	0 659	0.682	0 695	0 703	0 709	0 713	0 716	0 718	0 720	0 723	0 726	0 729	0 731	0 735	0 737	0 739
	0.4	0.60	0 640	0 655	0 663	0 667	0 671	0 673	0 675	0 676	0.678	0 679	0 681	0 683	0 684	0 686	0 687	0 688
0 50	0.0	0 50	0 632	0 707	0 756	0 791	0 816	0 837	0 853	0 866	0 877	0 894	0 913	0 933	0 945	0 971	0 985	1 000
	0 1	0 50	0.609	0.664	0 698	0 722	0 739	0 751	0 761	0 769	0 776	0 786	0 797	0 808	0 815	0 830	0 837	0 845
	0.2	0.50	0 587	0 628	0 652	0 668	0 679	0 688	0 694	0 699	0 704	0 710	0 717	0 724	0 728	0 736	0 741	0.745
	0 3	0 50	0 568	0 598	0 614	0 625	0 632	0 638	0 642	0 645	0 648	0 652	0 657	0 661	0 663	0 669	0 671	0 674
	0.4	0 50	0 550	0 571	0 582	0 589	0 594	0 598	0 600	0 602	0 604	0 607	0 609	0 612	0 614	0 617	0 619	0 620
0 40	0.0	0.40	0 525	0 603	0.658	0 698	0.730	0 756	0 777	0 795	0 810	0 834	0 861	0 890	0 909	0 951	0.975	1.000
	0 1	0 40	0.504	0 562	0 603	0 626	0 646	0 661	0 674	0 684	0 692	0 705	0 719	0 734	0 743	0 763	0 774	0 784
	0 2	0.40	0.485	0 528	0 555	0 573	0 586	0 595	0 603	0 609	0 614	0 622	0 630	0 639	0 644	0 655	0 661	0.667
	0 3	0 40	0 468	0 500	0.519	0 531	0 539	0.546	0 551	0 555	0 558	0 563	0 568	0 573	0 576	0 583	0 586	0 590
	0 4	0.40	0 453	0.476	0 489	0 497	0 503	0 507	0 510	0 513	0 515	0 518	0 521	0.524	0 526	0.530	0 532	0 535
0.30	0.0	0.30	0 406	0.478	0.532	0 575	0 610	0 640	0 665	0 686	0 705	0 737	0 773	0 815	0 844	0 912	0 953	1.000
	0.1	0.30	0.389	0 442	0.479	0.506	0 526	0 543	0 556	0 567	0 576	0 591	0 607	0 625	0 636	0 661	0 674	0 688
	0 2	0.30	0 374	0.413	0 439	0.456	0 469	0 480	0 487	0 494	0 499	0 508	0 517	0.526	0 532	0 544	0 550	0 557
	0 3	0 30	0.360	0 389	0.407	0.419	0 428	0 434	0 439	0 443	0 447	0 452	0 457	0 463	0 466	0 473	0 477	0 480
	0.4	0 30	0 348	0 369	0 382	0 390	0 396	0 400	0 403	0 406	0 408	0 411	0 414	0 418	0.420	0 424	0 426	0 429

limited to a value of about 0.7 or less except in pedigrees where there has been a very significant amount of close breeding. There is a legitimate place for the use of both pedigree and progeny test in rating a bull's real breeding worth and also room for attention to the bull's individuality. As the progeny test becomes more thorough and reliable we should of course give it relatively more attention in making our estimates. This means that pedigree and the bull's individuality will receive relatively less attention but we cannot now foresee any logical reason for neglecting them altogether, even in the most adequate of progeny tests possible.

What then is the number of daughters which must be tested before we can have an adequate progeny test of the sire? Obviously there is no number below which we must say the test is inadequate and above which we can say that the test is certainly correct. Our reliance in the progeny test should increase as the number of daughters increases, but at an ever-decreasing rate. At low values of *s* and *e* we may gain a very noticeable increase in the reliability of the progeny test by increasing the number of daughters even past eight or ten. At the other extreme with high values of both *e* and *s* there is little accuracy to be gained by increasing the number of daughters even past three or four. For what appear to be the most probable values for *s* and *e* only a little increase in accuracy is to be gained by including more than about five to eight daughters in the progeny test although of course one would want to base his estimate upon all that are available no matter how many that may be.

If a definite number must be adopted in order formally to define what a "proved sire" is, perhaps the number five, adopted by the Bureau of Dairy Industry, is as practical as any other. Certainly it is distinctly more accurate than a progeny test based on three or four except when the value of *e* is quite high. On the other hand the increase in accuracy which would be gained by requiring more than five tested daughters in order to "prove" a sire would be large only when the values of *e* and *s* are low. From the principles underlying the behavior of the progeny test, it must be quite obvious that we will occasionally encounter cases where a sire "proved" to be good in one herd will with equal certainty "prove" to be bad in another. Some sires will be "proved" to be poor ones merely because of chance variations or because they were used in a herd where the care and management given their daughters were not adequate.

The progeny test is rightly esteemed as one of the most valuable weapons which the breeder has at his command and everyone interested in the breeding of better livestock will do everything possible to promote its wider use, but we will save ourselves from some severe and perplexing disappointments if we keep in mind the principles on which the reliability of the test is based and thus know how much confidence to place in it.

THE INFLUENCE OF SWEETENED, FROZEN CREAM ON THE DEVELOPMENT OF SWELL IN ICE CREAM*

WALTER V. PRICE

Department of Dairy Husbandry, University of Wisconsin

The storage of milk fat for use in ice cream is a common procedure. It is usually held in frozen storage in the form of unsalted butter, butter oil, or low-acid cream. Within the past few years it has become common practice in the ice cream industry to add from 10 to 40 per cent of sugar to low-acid cream before it is placed in storage. The sweetened or non-sweetened cream is stored in tin cans, milk cans, and, occasionally, wooden barrels.

The use of non-sweetened frozen cream in ice cream causes certain changes in the physical characteristics and freezing properties of ice cream mix. According to Price and Whitaker (1), the basic viscosity of ice cream mix is increased and the whipping properties are decreased by the use of frozen cream.

The changes, which are caused in ice cream mix by the use of frozen cream, are almost identical to the changes which are caused by the use of butter instead of fresh cream. Whitaker (2) believes that the difference in the lecithin content of ice cream mix which contains cream and one which contains butter is responsible for these changes. He points out that replacing the lecithin, which was removed by the churning of the cream, by using buttermilk or egg yolk, both of which contain lecithin, restores the whipping properties of the butter mix.

Urbain and Miller (3) investigated the effect of freezing on egg white and egg yolk and found that the only significant change was the separation of lecithin from the egg yolk. They showed that the addition of sucrose, dextrose and levulose in concentrations of 1 part sugar to 10 parts of egg yolk prevented this separation, and that the sucrose was less effective, in equal concentrations, due to

---

* Received for publication September 15, 1930. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

the difference in its influence on the osmotic pressure of the mixture of egg yolk and sugar. This explanation is also supported by the evidence of a patent (4) which involves the use of salt for the preservation in frozen storage of the emulsifying properties of egg yolks for the manufacture of mayonnaise.

Since the emulsifying properties of egg yolk lecithin are altered by freezing and thawing, it is possible that the colloidal properties of cream may be altered by similar treatment. Urbain and Miller showed that mixtures of sugar and egg yolks prevented the separation of lecithin during freezing. The addition of sugar to cream, which is to be frozen and stored for subsequent use in ice cream, may also prevent those changes in the colloidal system of the cream that cause the decrease in the development of overrun of the mix.

A series of experiments were planned to test the effect of the addition of sugar to cream which was subsequently frozen, held, thawed, and then used to make ice cream.

THE EFFECT ON THE DEVELOPMENT OF SWELL OF USING CREAM, FROZEN FOR 48 HOURS, WITH AND WITHOUT SUGAR

A quantity of fresh pasteurized cream, containing 45 per cent fat, was divided into 4 lots. The first portion was stored at approximately 35°F. The second was frozen at −15°F. Sucrose was added to the third portion at the rate of 1 part of sugar to 10 parts of cream, by weight. Invert syrup, which was made by inverting sucrose with citric acid, according to the method described by Jordan (5), was added to the fourth portion of the cream at the rate of 1.33 parts of syrup to 10 parts of cream by weight. This amount of syrup is equivalent to 1 part of sugar to 10 parts of cream. The third and fourth lots were also frozen at −15°F. All four lots were held in well-tinned ice cream cans.

The storage period lasted for forty-eight hours and then the frozen lots were placed in cold water and allowed to remain undisturbed until all ice disappeared.

The effects of the storage treatments on the appearance of the four lots of cream were clearly evident. The first lot, which had been held at 35°F., was unchanged. The second portion, which contained no sugar, was granular due to the presence of small aggregates of milk fat, and minute particles of serum solids. The fat separated from the mix, in which this cream was used, in the form of oil during the pasteurizing period and was only incorporated by the homogenizing process. The third portion resembled the second but the aggregates of fat were smaller and less numerous and there was only a slight separation of milk fat during pasteurization. The appearance of the fourth portion was practically identical to that of the normal unfrozen cream. There were no aggregates of fat visible to the naked eye and no fat separation.

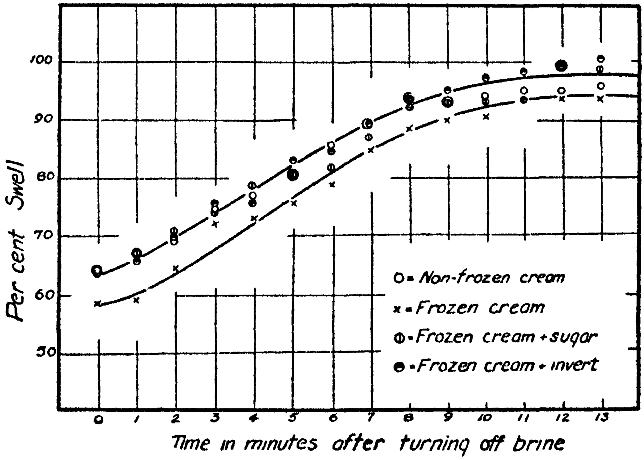


FIG. 1. THE INFLUENCE OF SWEETENED CREAM, FROZEN FOR 48 HOURS, ON THE DEVELOPMENT OF SWELL OF ICE CREAM

Four mixes were made which differed from each other only in that they contained a different one of the four lots of cream. Each mix contained identical quantities of sugar and invert syrup, which together furnished 15 per cent of the weight of the mixes. Fat, skimmilk solids and gelatin, composed 12, 11 and 0.25 per cent, respectively of each mix. The skimmilk solids required, in excess of the quantity in the cream, were supplied by dry skimmilk. The mixes were pasteurized at 145°F. for thirty minutes, homogenized at 3000 pounds pressure and then cooled to approximately 40°F. and aged at that temperature for sixteen to eighteen hours.

The procedure was repeated with four lots of cream during the period from April 11 to May 31, 1930. It is possible that the results might be different at other seasons of the year when the lecithin content of the milk might show the influence of other conditions.

The results of the different treatments, to which the four lots of cream were subjected, upon the freezing properties of the ice cream mixes are shown in figure 1 and table 1.

TABLE 1

The effect of sweetened and non-sweetened frozen cream on the development of swell

TREATMENT OF CREAM	KIND OF SUGAR	RATIO OF SUGAR TO CREAM	STORAGE PERIOD OF CREAM	NUMBER OF TRIALS	TIME REQUIRED TO DEVELOP 90 PER CENT SWELL	MAXIMUM SWELL OBTAINABLE*
			hours		*minutes*	*per cent*
Not frozen	None		48	4	8	96
Frozen	None		48	4	9	94
Frozen	Sucrose	1:10	48	4	8	99
Frozen	Invert	1 33:10	48	4	8	101
			days			
Frozen	None		14	3	9	95
Frozen	Sucrose	1.5:10	14	3	7	100
Frozen	Invert	1:10	14	3	7	102
Frozen	Sucrose	4:6	14	3	7	100

* "Maximum swell obtainable" with proper consistency for drawing from freezer.

The mixes containing non-sweetened, frozen cream were slightly inferior in whipping properties to those mixes which contained non-sweetened, unfrozen cream. The swell development of the non-sweetened, unfrozen lot of cream practically coincided, during the first nine minutes of the whipping period, with that of those mixes which contained the sweetened frozen cream.

Those changes in the freezing properties of the mix, that were caused by the use of non-sweetened frozen cream, were eliminated by the addition of either sucrose or invert syrup to the cream before it was placed in frozen storage.

THE EFFECT ON THE DEVELOPMENT OF SWELL OF USING CREAM, FROZEN FOR 14 DAYS WITH OR WITHOUT ADDED SUGAR

The difference in development of swell between the mixes, which contained frozen and unfrozen cream without sugar, was smaller than the differences noted by Price and Whitaker (1). This disagreement was believed to be due to the difference in the length of time the cream remained frozen. A second series of experiments were planned to permit a longer storage period.

In this second series of experiments, a quantity of fresh, pasteurized cream, containing 47 per cent fat was divided into four portions. The first portion was not altered. To the second was added 1.33 parts of invert syrup for each 10 parts of cream by weight. To the third was added 1.5 parts of sucrose for each 10 parts of cream. The fourth portion was separated to produce 60 per cent cream and sucrose was added at the rate of 40 parts of sugar to 60 parts of cream.

All four lots of cream were placed in well-tinned ice cream cans and frozen at −15°F. After fourteen days they were thawed by immersion in cold water.

The appearance after melting of the sweetened cream in each instance was smooth and practically free from granules of milk fat. In the first series of experiments, the appearance of the cream to which the sucrose had been added, was not as smooth as that of the cream which contained invert syrup. This was apparently caused by the difference in the freezing point depression induced by the sucrose and the inverted sugar, because the granular condition was avoided by the addition of larger amounts of sucrose in this second series of experiments. That portion of the cream which contained 40 per cent sucrose was similar in body to sweetened condensed milk. It had a slightly translucent appearance and a very pleasing flavor. The non-sweetened portion presented the usual granular appearance and was the only lot to show fat separation during the pasteurizing of the mix in which it was used.

Four mixes were made which differed from each other only in that they contained a different one of the four lots of cream.

They were identical in composition to the mixes of the first series of experiments and were made and processed in the manner which has been described.

This procedure was repeated during June, 1930 until three different lots of cream had been so divided and treated.

The results of the freezing operations are summarized and presented in figure 2 and table 1.

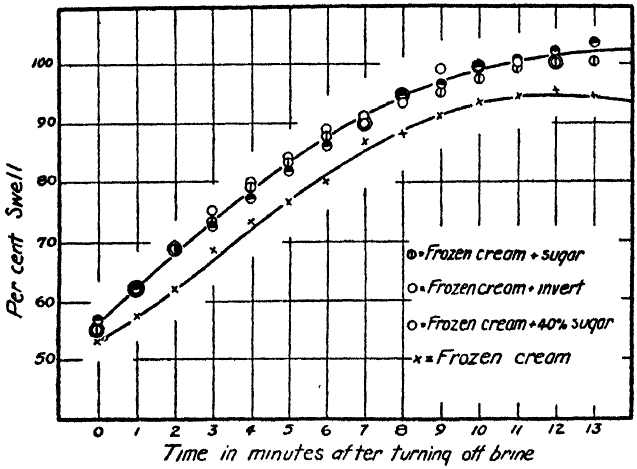


FIG. 2. THE INFLUENCE OF SWEETENED CREAM, FROZEN FOR 14 DAYS, ON THE DEVELOPMENT OF SWELL OF ICE CREAM

The swell developed by the mixes containing the sweetened, frozen cream was practically identical, regardless of the sugar content of the cream. The swell obtained in the mix containing the non-sweetened, frozen cream, developed more slowly and attained a lower maximum.

Here again is evidence of the tendency of sugar to prevent that change in frozen cream which causes a decrease in the development of swell of the ice cream mix in which it is used.

Observations indicated that the quality of the ice cream was not altered by the difference in the treatment of the frozen cream.

THE EFFECT ON THE DEVELOPMENT OF SWELL OF USING EGG YOLK FROZEN WITH AND WITHOUT SUGAR

A third experiment was planned to determine the effect on the development of swell of the mix of the use of egg yolk frozen with sugar to prevent the separation of lecithin.

The yolks of 18 eggs were mixed together and then divided into two equal portions. One portion was not altered but to the second was added 1.33 parts of invert syrup for each 10 parts of egg yolk by weight. The yolks were then placed in well-tinned cans and frozen for forty-eight hours at −15°F. The containers were then placed in cold water until all ice disappeared.

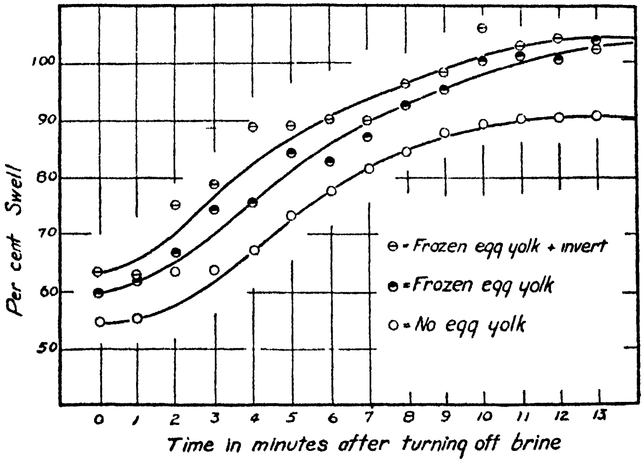


FIG. 3. THE INFLUENCE OF EGG YOLKS, FROZEN WITH INVERT SIRUP, ON THE DEVELOPMENT OF SWELL OF ICE CREAM

The appearance of the sweetened egg yolks was not altered by this treatment, but the non-sweetened lot contained small, dark orange-colored jelly-like masses which could not be reincorporated by agitation.

An ice cream mix of the usual composition was made in which butter served as the source of milk fat and dry skimmilk as the source of skimmilk solids in order that no appreciable quantity of lecithin could be present in the milk products. It was divided into three 50-pound portions. To one lot was added the non-sweetened egg-yolk. To the second was added the sweetened egg-yolk. The third portion remained unaltered. Each portion was then pasteurized, homogenized, cooled and aged in the usual manner which has been described.

The three mixes were then frozen and the results are shown in figure 3.

The swell developed by the mix which contained the sweetened egg-yolk was slightly higher than that of the mix which contained the non-sweetened egg-yolk. Both of these mixes possessed distinctly better whipping properties than the egg-free mix.

The process of freezing and thawing the egg-yolks caused a change in their influence on the development of overrun. The addition of sugar to the yolks, before they were frozen, decreased the intensity of this effect. The work of Urbain and Miller suggests that these results were due to the influence of the sugar on the lecithin of the egg-yolk.

CONCLUSIONS

Ice cream mix which contains sweetened frozen cream as the only source of milk fat possesses better whipping properties than mix which is made with non-sweetened frozen cream.

Invert syrup and sucrose are equally effective for sweetening cream for storage if the concentrations of each cause approximately the same freezing point depression in the storage cream.

The available evidence indicates a possible relationship between lecithin and the swell development of ice cream mix.

REFERENCES

(1) Price, Walter V., and Whitaker, Randall. 1931. Dry skimmilk in ice cream. New York Agric. Exper. Sta., Cornell University, Bulletin 516.

(2) Whitaker, Randall 1930 The influence of the use of butter on the freezing properties of ice cream mix. Jour. Dairy Sci., xiii, no. 1, 1.

(3) Urbain, O. M., and Miller, J. M. 1930 Relative merits of sucrose, dextrose and levulose as used in the preservation of eggs by freezing. Indust. and Eng. Chem., xxii, no. 4, 355.

(4) Rector, Thomas M. Preserving egg yolks. U. S. patent No. 1,744,575. January 21, 1930.

(5) Jordan, S. 1924 Invert sugar, its manufacture and uses. Indust. and Eng. Chem., xvi, 307–310.

FAT SOLUBLE VITAMINS

XXXIII. THE DETERMINATION OF VITAMIN A AND ITS STABILITY IN BUTTER FAT TO ULTRA VIOLET RADIATIONS*

H. STEENBOCK AND ALICE M. WIRICK

WITH THE COOPERATION OF BLANCHE M. RIISING

Department of Agricultural Chemistry, University of Wisconsin, Madison

In making use of the ultraviolet rays as an antirachitic activating agent, it is necessary, of course, to consider harmful or destructive effects which may be produced simultaneously as the food materials are exposed. It is well known that protein substances become coagulated, that enzymes are destroyed and that even starches acquire a very distinctive taste upon long exposure. Zilva (1) showed that vitamin C in decitrated lemon juice was not destroyed by exposure to the radiations of a quartz mercury vapor lamp for six to eight hours; vitamin B in autolyzed yeast, after being exposed for six hours, apparently was not destroyed, but vitamin A in butter showed itself to be much more labile to this treatment, because after an exposure of eight hours, the butter fat, fed at a level of 2 per cent did not prevent the incidence of ophthalmia in rats. Spinka (2) reported that ultraviolet light did not destroy vitamin B in yeast nor vitamin C in lemon juice, but treated butter fat was found toxic unless air was excluded. Zilva (3) reported that the death of Spinka's mice possibly was not due to toxicity but to destruction of vitamin A. These deductions were based upon Zilva's experience with rats in his previous paper (1). Later Zilva (4) showed that this destruction was due to ozone generated during the course of radiation. When the radiation was carried out in CO_2 vitamin A was not destroyed. Peacock (5) and Holmes and Pigott (6) also referred to vitamin A destruction by ultra violet. Titus, Hughes, Henshaw, and Fitch (7) showed that in the irradiation of milk, vitamin A con-

---

* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station. Received for publication October 2, 1930.

tained therein was materially decreased. Drummond (8) has been reported as having pointed out the possibility of vitamin A destruction in the course of activation of milk, and also of having called attention to the well known changes produced in palatibility. Steenbock and Coward (9) pointed out that both vitamins A and B showed remarkable stability to ultraviolet treatment. Hogan and Hunter (10) found that while vitamin B was not destroyed, the heat stable factor often accompanying it was destroyed. Supplee and Down (11) found that milk could be activated either in the dry or liquid form by a short time exposure with careful control of other conditions without destruction of vitamin A or induction of toxic effects. Steenbock and Black (12) showed that olive oil excessively irradiated lost the antirachitic activity originally induced. With cod liver oil they likewise found that after long continued exposure the oil became totally devoid of antirachitic potency. This probably explains why it is impossible (13) to increase materially the antirachitic potency of cod liver oil even though it contains activatable substances. Presumably the compounds already activated are destroyed in the same measure as the unactivatable substances become potent. Willimott and Wokes (14, 15) found that in cod liver oil destruction of vitamin A continued after irradiation.

The above resumé of the experience of various investigators shows very definitely that the induction of antirachitic activation in food materials by means of ultraviolet irradiation must be carried out very carefully. Especially is this true of such foods as milk, which due to their content of labile proteins and fats are probably as susceptible to harmful agents as any with which we have to deal.

EXPERIMENTAL

In the various June butter fat samples irradiated as described in the preceding paper of this series (16), determinations for vitamin A content were made with samples which had been irradiated for 30 minutes, 60 minutes, and 120 minutes. As will be recalled, these samples in general were irradiated with a quartz mercury vapor lamp of the Alpine Sun type, exposing the butter fat at depths of 0.08, 0.16, 0.32, 0.64, and 1.28 cm. in bright pie tins, 7¾ inches in diameter. Due to the fact that Zilva (4) reported failure of destruction of vitamin A when the irradiation was carried out in an atmosphere of CO_2, additional samples of butter fat were prepared which were irradiated at a depth of 0.08 cm. in the presence of nitrogen, carbon dioxide, and hydrogen. These exposures were made on 9.3 cc. of butter fat measured out into a small tin pan 10.8 cm. square. This pan was slipped into a small galvanized iron chamber, provided with a quartz window. The chamber was then closed by means of a plate clamped down, air tight, on a rubber gasket. The air was displaced from the chamber by the aforementioned gases as measured out from tanks into a large rubber balloon. The carbon dioxide and hydrogen were used as obtained commercially. The nitrogen was further purified by passing it through alkaline pyrogallol.

The content of vitamin A in the butter fats was determined on the premises outlined by Steenbock and Coward (9). We used animals produced in our general stock at approximately 40 grams in weight and approximately twenty days old. The basal ration used was composed of purified casein 18, agar 2, salts 40, (17) 4, yeast 8, dextrinized starch 68. This ration, in order to endow it with antirachitic properties in excess, was irradiated in 250 grams quantities in a pan measuring 2 feet square for a period of 30 minutes. It was stirred once after 15 minutes' exposure. The irradiation was carried out at a distance of approximately 18 inches, with the same lamp as that used for the treatment of butter fat. After the animals had been on this ration for a sufficient period of time to develop ophthalmia,—which was usually in the neighborhood of five weeks, they were taken for test. At first, we used the animals by litters. When the majority of the rats in a litter had developed ophthalmia, the entire litter, irrespective of the condition of the others, was taken. However, we soon discovered that there was no greater uniformity in the reaction of individuals from the same litter than from different litters. Accordingly, for most of the experiments the animals were taken without regard to litters, as soon as they had developed the desired degree of ophthalmia. This ophthalmia was designated as stage 3 which was characterized by a severe erythema accompanied by bareness of eyelids, occasional laceration with excessive exudate sometimes causing closure of the eyes. In order to follow the progress of the disease in its development, as well as its regression, the ophthalmias were classified by number according to severity. The progressive stages are:

0—Normal
1—Accumulation of exudate, dry or moist
2—Swollen eyelids with or without incipient bareness
3—Erythema of conjunctiva, with or without bareness, laceration or closure
4—Purulency, accompanied by closed eyes with severe swelling and occasional blindness

and the regressive changes:

3—Absence of purulency, but persistence of erythema, bareness, swelling and exudate with eyes open or closed
2—Absence of erythema, but eyes still swollen, cornea cloudy, presence of exudate, eyelids bare
1—Absence of acute reaction, but eyes slightly dirty or watery with or without protrusion of cornea and presence of scar tissue
0—Normalcy; absence of exudate, bareness of lids and scar tissue

Inasmuch as Evans and Bishop (18) have reported the persistence of cornified cells in the vaginal smear of females as a far more sensitive symptom of vitamin A deficiency, than ophthalmia, we have examined some of our animals using their technique. Our experience is limited to 13 animals. These were started on a vitamin A free diet at an age of 21 to 22 days. They developed ophthalmia after four to five weeks on this ration. When examined for the presence of cornified cells in the vaginal smear after they had been on the ration for 37 to 41 days they were still relatively immature as the vaginal orifice was found closed. They were kept under observation from 5 to 25 days, after which periods all except one had died as a result of the dietary deficiency. When the observations were started, 4 of the animals were in stage 2 ophthalmia, 6 were in stage 3, and 3 were in stage 4. With these severe ophthalmias only 5 females showed the presence of cornified cells. Most of them had very scanty smears and later showed a definite cyclic rhythm. Shortly before the cessation of observations, 8 females had cornified cells. From this experience, limited though it is, we are inclined to believe that data on ophthalmia are more satisfactory than those obtained by Evans' technique (18). If in our experimets we made the mistake of using the females at too early an age, taking them at this age has much to commend it because when the animals are taken later, the storage reserves are frequently so high that depletion occurs relatively slowly.

For the determination of vitamin A the butter fat was for the most part fed incorporated in the ration. This procedure was felt to be justified because we obtained the total consumption records throughout the period of feeding. We have, therefore, a check on the amounts of butter fat received by the animals in the different groups. However, in some of our experiments, namely in those in which we tested out the effect of gases other than air on the preservation of vitamin A, the butter fat was measured out in small quantities in dishes,—the basal ration in all cases being fed ad libitum. In terms of percentage of the ration, butter fat irradiated for 30 minutes was fed at levels of 0.25, 0.5, 1.0 and 2.0 per cent; irradiated for 60 minutes at 2 per cent, and irradiated for 120 minutes at 1, 2, 4 and 8 per cent.

The results on ophthalmia are not all presented for the simple reason that 4 out of the series of 9, happened to be run at such a level that they alone bring out the approximate border line of persistence as contrasted with the destruction of vitamin A. In table 1, as in the other tables, the vacant line represents the line of reference with respect to the condition of the ophthalmia in the animals after two weeks on the supplemented diet.

Table 1 shows that butter fat irradiated for 30 minutes incorporated in the ration at 0.5 per cent level had appreciable quantities of its vitamin A destroyed when irradiated at depths of 0.08 and 0.16 cm. When irradiated at greater depths the rapidity in the cure of ophthalmia was not different from that effected with the feeding of untreated butter fat at the same level.

TABLE 1

Cure of ophthalmia with butter fat irradiated 30 minutes 0.5 per cent in ration

WEEKS	DEPTH OF BUTTER FAT WHEN IRRADIATED																													NON-IRRADIATED				
	0.08 cm.							0.16 cm.								0 32 cm.							0.64 cm.			1.280 cm.								
	Lot 45*			Lot 50				Lot 46				Lot 52				Lot 47				Lot 54			Lot 30			Lot 29				Lot 22				
0	0	3	3	3	3	2	3	0	3	0	2	3	3	2	3	3	1	3	2	3	3	3	1	3	3	1	4	3	3	2	4	3	3	3
1	0	2	3	3	3	1	3	0	1	0	2	3	3	2	3	1	1	2	1	3	3	1	0	1	3	1	3	0	1	1	3	1	3	1
2	0	3	3	3	0	3	3	0	1	0	2	0	3	2	3	0	1	†	1	1	0	1	0	1	0	0	1	0	1	†	3	1	1	1
3	†	1	3	3	0	3	3	†	†	0	2	3	3	2	3	†	0		1	1	†	1	2	1	0	0	1	0	1		3	1	1	3
4		2	†	3	0	3	2			0	2	3	3	†	3		0		1	1		1	†	†	1	0	1	3	1		1	1	1	1
5		†		3	0	1	3			0	1	3	3		†					1		1			0	0	3	†	1		1	1	1	1
6				3	3	3	†			0	3	3	†							†		1			0	0	†		1		1	1		1
7				3	3	†				†	†	3										1									1	1		
8				3	3																													

* Each column carries the data for an individual rat.

Key to ophthalmia tables. 4 = purulency accompanied by closed eyes with severe swelling and occasional blindness. 3 = absence of purulency, but persistence of erythema, bareness, swelling and exudate with eyes open or closed. 2 = absence of erythema, but eyes still swollen, cornea cloudy, presence of exudate eyelids bare. 1 = absence of acute reaction, but eyes slightly dirty or watery with or without protrusion of cornea and presence of scar tissue. 0 = normalcy: absence of exudate, bareness of lids and scar tissue.

Table 2 shows that when the butter fat was fed at a 1 per cent instead of a 0.5 per cent level as in table 1 the ophthalmias were cured in all the groups with equal efficiency. It is to be emphasized that little importance is to be attached to the persistence of stage 1 of ophthalmia because scar tissue is usually absorbed with great difficulty. Regressive stage 1 is, therefore, to be taken as of practically the same value as stage 0.

TABLE 2

Cure of ophthalmia with butter fat irradiated thirty minutes, 1.0 per cent in ration

WEEKS	DEPTH OF BUTTER FAT WHEN IRRADIATED																				NON-IRRADIATED			
	0 08 cm.							0 16 cm.				0 32 cm			0 64 cm.				1 28 cm					
	Lot 13				Lot 19			Lot 14				Lot 15			Lot 16				Lot 17		Lot 18			
0	0	3	3	3	1	0	3	2	3	3	1	2	3	3	2	3	4	3	4	3	2	3	4	3
1	0	2	3	0	0	0	3	3	0	2	0	3	1	0	0	0	3	0	3	0	1	1	1	2
2	†	1	1	0	0	0	1	†	0	1	0	1	1	0	†	0	3	0	3	0	1	1	1	0
3		1	1	0	0	0	0		0	1	0	1	1	0		0	1	0	1	0	1	1	†	0
4		1	1	0	0	0	0		0	1	0	1	1	0		0	1	0	1	0	1	1		0
5		1	1	0	0	0	0		0	1	0	1	1	0		0	1	0	1	0	1	1		0
6		0	1	0					0	1	0	0	0	0		0	1	0	1	0	0	1		0

TABLE 3

Cure of ophthalmia with butter fat irradiated two hours, 2.0 per cent in ration

WEEKS	DEPTH OF BUTTER FAT WHEN IRRADIATED																											NON-IRRADIATED		
	0 08 cm								0 16 cm							0 32 cm				0 64 cm				1 28 cm						
	Lot 7				Lot 31			Lot 37	Lot 8				Lot 32			Lot 9				Lot 10				Lot 11				Lot 12		
0	2	2	1	0	3	4	3	3	3	2	3	0	3	2	0	3	3	3	0	3	3	0	3	3	4	3	3	3	4	3
1	2	3	3	0	3	3	3	3	2	2	2	0	3	1	3	1	1	3	0	0	3	0	0	0	1	0	1	0	3	1
2	2	1	3	0	3	1	3	3	3	†	†	3	†	†	3	2	0	0	0	0	1	0	0	0	3	0	1	0	3	1
3	†	†	†	†	†	†	†	†	3			2			3	1	0	0	0	0	1	0	0	0	1	0	1	0	1	1
4									†			1			†		0	0	0	0	1	0	0	0	1	0	1	0	1	1

Table 3 shows that more destruction resulted when the butter fat was irradiated for two hours at depths of 0.08 and 0.16 cm. than at the greater depths. When the amount of butter fat in the ration was increased from 2 to 4 per cent (table 4) this greater destructive action at the aforementioned depths was still definitely demonstrable.

We have also noted that when the butter fat was exposed for 60 minutes instead of 120 and fed at a 2 per cent level, the ophthalmias were cured inside of two weeks to stage 0 and stage 1, even with those samples which had been exposed at a depth of 0.08 and 0.16 cm. The detailed results of these trials are not presented.

The above results, together with those presented in the preceding paper (16), bring out convincingly that while antirachitic activation is effected more pronouncedly with the butter fat exposed in thin layers, concomitantly greater vitamin A destruction also results. The significance of the observed destruction with 30 minutes' exposure at a depth of 0.16 cm. must not, however, be over emphasized, because when the amount of butter fat in the ration was doubled, sufficient vitamin A was still present to effect a rapid cure of ophthalmias.

TABLE 4

Cure of ophthalmia with butter fat irradiated two hours, 4 per cent in ration

WEEKS	DEPTH OF BUTTER FAT WHEN IRRADIATED																							
	0.08 cm.							0 16 cm.						0 32 cm.							0 64 cm.			
	Lot 23			Lot 38		Lot 42		Lot 24				Lot 43		Lot 25				Lot 44			Lot 26			
0	3	3	1	3	3	3	1	3	3	2	2	3	0	3	3	2	3	3	1	0	3	3	3	3
1	2	1	1	3	3	3	3	1	1	2	3	3	0	3	1	1	3	3	1	0	2	3	1	3
2	3	1	2	†	†	3	2	2	2	2	2	3	0	2	1	1	0	0	0	0	1	3	1	0
3	3	1	2			3	3	1	3	2	2	3	2	1	1	1	0	0	0	0	1	0	1	0
4	†	†	†			3	2	1	†	3	†	3	3	1	1	1	0	0	0	0	3	0	1	0
5						1	2	†		3		†	3	1	1	0	0				1	0	0	0
6						3	3			†			3	1	0	0	0				2	0	0	0
7						†	†						3											
8													3											
9													3											
													†											

Besides using the cure of ophthalmia as our criterion for vitamin A content of the butter fat samples, we also kept records of the growth of the animals before and after supplementation of the basal ration. The animals were weighed once a week and their growth curves plotted as presented in charts 1 to 5. In chart 1 are presented the results obtained with butter fat irradiated at

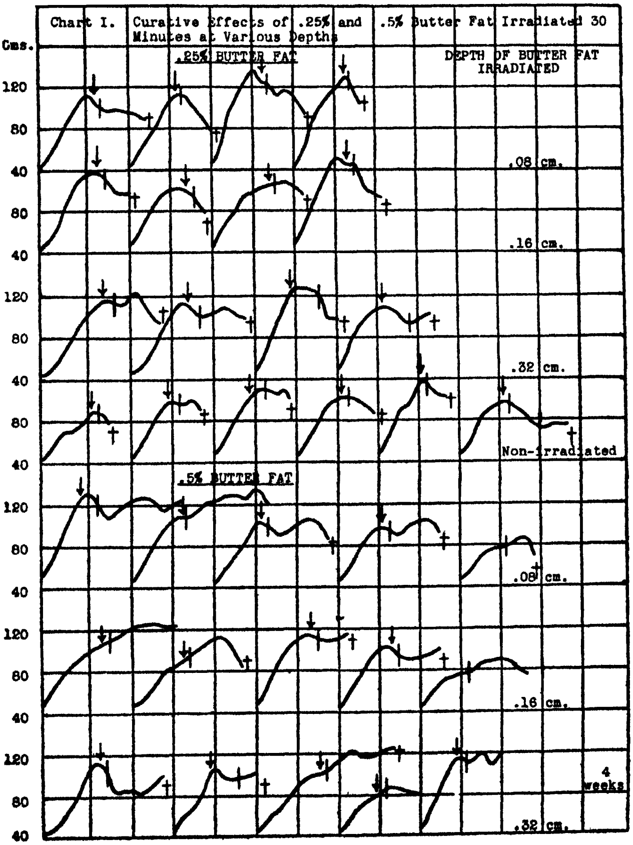


CHART 1

↓ incidence of ophthalmia, | change of ration, † death

depths of 0.08, 0.16 and 0.32 cm. when fed at levels of 0.25 and 0.5 per cent of the ration. We have not presented data on the cure of ophthalmia at the 0.25 per cent level of intake because this was too low an intake to effect a cure even with the butter fat irradiated at the greater depths, but we have included the growth curves at this level of intake to bring out that growth was not markedly, if at all, inferior to that in the 0.5 per cent of fat. It will be recalled that on 0.5 per cent of fat, we obtained rapid cure of ophthalmia with butter fat irradiated at a depth of 0.32 cm., as contrasted with 0.08 and 0.16 cm. Inasmuch as the differences in growth do not bring out these relations we were at this point inclined to believe that the ophthalmia method of vitamin A determination was to be preferred to the growth method. It will be noted that in the charts not all of the growth curves of the animals on which we have ophthalmia data are presented. Those shown are in every way representative of the groups as a whole.

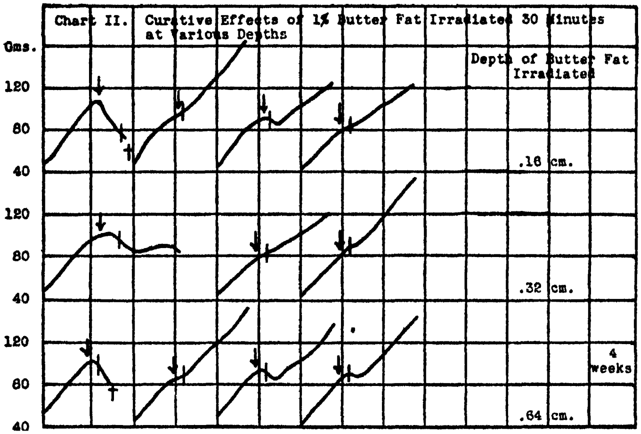


CHART 2

↓ incidence of ophthalmia, | change of ration, † death

In chart 2 are presented data with butter fat fed at a level of 1 per cent irradiated for 30 minutes at depths of 0.16, 0.32, 0.64 cm. At this level of intake the growth curves are in every way comparable to the results on ophthalmia.

In chart 3 are shown the growth curves comparing butter fats irradiated in depths of 0.08, 0.16, and 0.32 cm. for periods of one as contrasted with two hours, fed at a 2 per cent level. When the butter fat was irradiated for one hour, growth in the animals was very promptly restored at all depths; but, when irradiated for two hours, growth was restored only with that which had been irradiated at 0.32 cm. Comparing these results with our ophthalmia data, it is significant that in this case the growth data are in

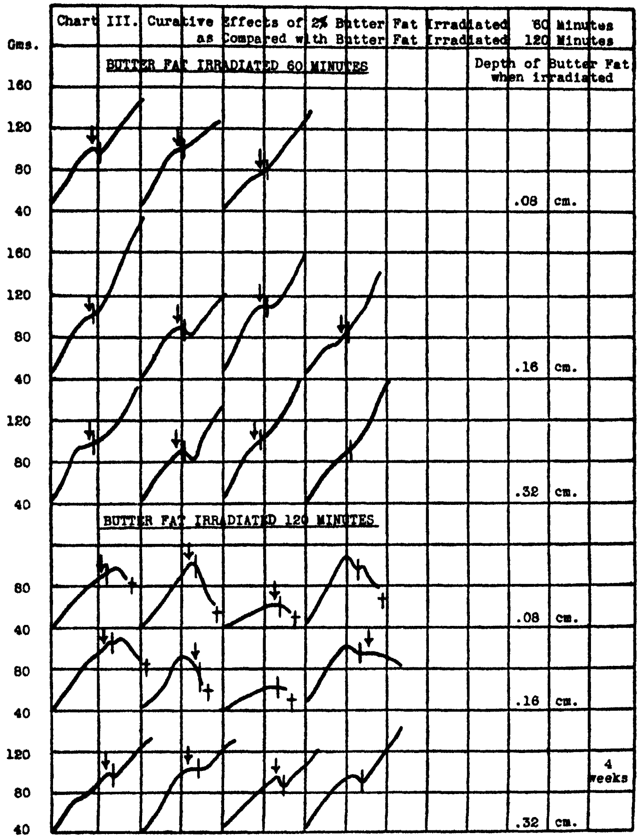


CHART 3

↓ incidence of ophthalmia, | change of ration, † death entire accord with our results on ophthalmia. By way of explaining this difference, as compared with our observations in the groups previously discussed, we are inclined to believe,—although the data selected for publication in this paper do not prove the point—that the growth observations harmonize with the cure of ophthalmia only when the loss in weight or the maintenance of constant weight previous to the change of the diet has been

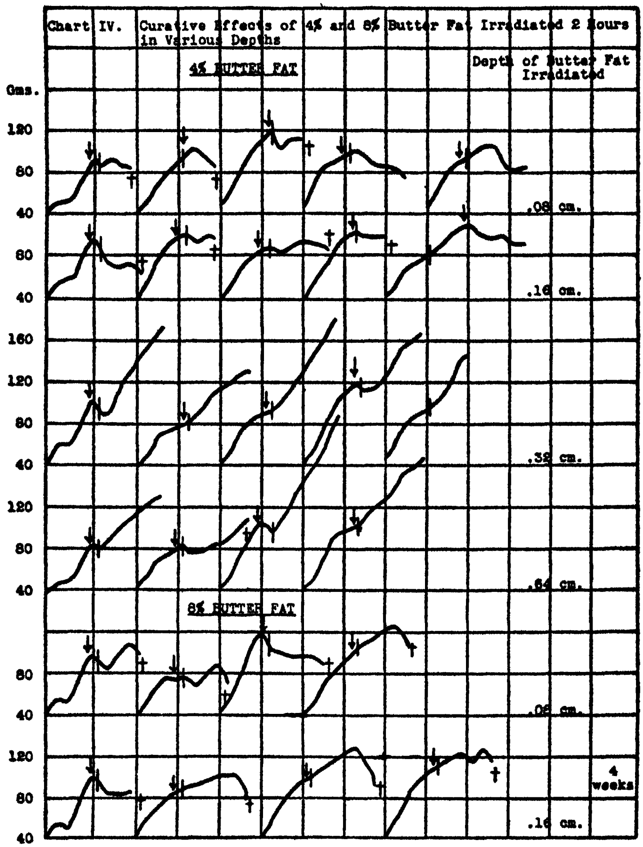


CHART 4

↓ incidence of ophthalmia, | change of ration, † death

TABLE 5

The average daily consumption by individual animals during the first two weeks or until death when the animal died sooner

0.25 per cent butter fat irradiated 30 minutes

	DEPTH OF BUTTER FAT WHEN IRRADIATED					NON-IRRADIATED
	0.08 cm	0.16 cm	0.32 cm	0.64 cm	1.28 cm	
	Lot 51	Lot 53	Lot 55			Lot 49
	grams	*grams*	*grams*	*grams*	*grams*	*grams*
	5.6	4.4	9.3			4.3
	5.0	4.6	6.4			6.2
	6.4	5.6	4.3			7.7
	3.2	3.0	6.7			8.4
						5.9
						3.7
Average per rat . .	5.1	4.4	6.7			5.5

0.5 per cent butter fat irradiated 30 minutes

	0.08 cm		0.16 cm		0.32 cm		0.64 cm	1.28 cm	NON-IRRADIATED
	Lot 45	Lot 50	Lot 46	Lot 52	Lot 47	Lot 54	Lot 30	Lot 29	Lot 22
	8.9	6.9	7.1	8.4	8.5	4.7	6.1	4.9	2.9
	4.7	7.1	7.3	7.4	8.9	7.3	5.0	5.8	7.3
	6.7	4.6	6.9	5.4	4.0	7.9	8.1	4.5	8.4
		5.1	5.0	6.0	5.9			7.9	4.1
									7.4
Average per rat . . .	6.3		6.7		6.7		6.4	5.8	6.0

1.0 per cent butter fat irradiated 30 minutes

	0.08 cm		0.16 cm	0.32 cm	0.64 cm	1.28 cm	NON-IRRADIATED
	Lot 13	Lot 19	Lot 14	Lot 15	Lot 16	Lot 17	Lot 18
	3.8	5.0	3.1	3.2	1.9	4.4	6.8
	6.6	5.4	8.1	6.7	7.5	8.1	6.4
	4.9	3.9	6.4	7.4	5.7		5.0
	8.2		7.4		6.4		7.8
Average per rat....	5.4		6.3	5.8	5.4	6.3	6.5

2.0 per cent butter fat irradiated 120 minutes

	0.08 cm			0.16 cm		0.32 cm	0.64 cm	1.28 cm	NON-IRRADIATED
	Lot 7	Lot 31	Lot 37	Lot 8	Lot 32	Lot 9	Lot 10	Lot 11	Lot 12
	4.9	7.4	5	5.4	6.6	7.6	9.3	6.3	8.1
	3.5	4.9		2.6	2.6	6.6	5.5	8.4	5.4
	3.7	5.2		4.1	6.1	5.8	6.9	6.1	7
	1.4			6.2		7.9	7.1	8.8	8.1
Average per rat....	4.5			4.8		7.0	7.2	7.4	7.2

TABLE 5—*Concluded*

	DEPTH OF BUTTER FAT WHEN IRRADIATED									NON-IRRADIATED
	0.08 cm.			0.16 cm.		0.32 cm.		0 64 cm.	1 28 cm.	
4 0 per cent butter fat irradiated 120 minutes										
	Lot 23	Lot 38	Lot 42	Lot 24	Lot 43	Lot 25	Lot 44	Lot 26		
	gms.	*gms.*	*gms.*	*grams*	*grams*	*grams*	*grams*	*grams*		
	5.0	4.8	5.9	3.9	7.6	7.1	8 6	7.5		
	5.6	3.1	7 7	4.6	6 6	7 9	10.4	5		
	4 8			5 9		7.1	8.4	9		
				5 3		7 8		8		
Average per rat....	5 3			5 7		8 2		7 4		

N.B. To compare with ophthalmia data (tables 1 to 4), tables5 should be read taking each column in order from left to right. This will give the consumption corresponding for each rat.

of short duration. This is in accordance with the observations of Macy and co-workers (19).

Chart 4 shows that with the butter fat irradiated for two hours, fed at 4 and 8 per cent of the diet, growth was not resumed by the animals, where the butter fat had been exposed at depths of 0.08 and 0.16 cm. At greater depths, namely, 0.32 and 0.64 growth was reinitiated promptly even at the 2 per cent level of intake. This is again in entire harmony with our observations on ophthalmia, which presentation in the charts, however, is limited to the 4 per cent level of intake. The data of the 8 per cent level of intake are not presented in order to conserve space.

As previously stated, consumption records were kept in all of the foregoing experiments in order to allow us to check the intake of butter fat. In the course of our experiments we have frequently had occasion to feed a dietary supplement in portions separate from the basal ration. Under many conditions we have found this entirely satisfactory, but in other cases, we have been led to doubt the advisability of such a procedure because it gives the experimenter no guarantee that the basal ration is consumed in sufficient amounts unless this is checked up also. We have, therefore, felt that records of the total food intake are far more satisfactory. For the reader's convenience and assurance of validity of deductions the records of food consumption for the first two weeks have been presented in table 5. While data were accumulated over the entire experimental period, it was thought sufficient to present only the first two weeks because with a satisfactory consumption for this period the animal should have responded to the dietary supplement if it contained a sufficiency of vitamin A.

TABLE 6

Cure of ophthalmia with butter fat irradiated in air nitrogen, carbon dioxide, and hydrogen

80 mgm. daily

WEEKS	IRRADIATED IN AIR						IRRADIATED IN NITROGEN					IRRADIATED IN CO_2						IRRADIATED IN HYDROGEN						NON-IRRADIATED					
	Lot 64						Lot 65					Lot 68						Lot 70						Lot 56					
0	3	3	3	3	3	3	3	3	3	3	3	4	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
1	3	3	3	3	3	3	3	3	1	3	3	1	3	1	3	3	3	2	3	3	3	2	2	0	1	0	0	0	0
2	†	2	3	3	3	3	3	3	2	3	†	3	3	0	3	3	3	1	3	2	2	†	2	0	0	0	0	0	0
3		2	3	†	†	†	†	3	0	3		†	2	0	3	3	3	1	3	3	1		2						
4		†	†					3	0	3			1	†	†	3	3	1	3	†	†		1						
5								3	0	3			1			3	3	1	3				†						
6								†	0	2			†						†										
7									0	1																			
8										1																			
										†																			

320 mgm. daily

WEEKS	IRRADIATED IN AIR				IRRADIATED IN NITROGEN						IRRADIATED IN CO_2						IRRADIATED IN HYDROGEN					NON-IRRADIATED					
	Lot 66				Lot 67						Lot 69						Lot 71					Lot 72		Lot 59			
0	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	3
1	3	3	3	3	2	3	1	3	0	3	0	3	3	2	3	1	3	0	3	1	1	1	1	0	1	0	0
2	3	3	3	2	0	0	0	1	0	3		2	1	0	1	0	2	0	1	0	0	1	1	0	1		
3	3	2	3	2				1		1		0	1		1	0	2	0	1								
4	2	†	†	2									1				1										
5	3			†													1										
6	3																										
	†																										

Comparing the results of our previous experiments (16) with the present, it appears that, approaching the point of maximum activation of butter fat, there becomes incident the destruction of demonstrable amounts of vitamin A. To determine if this is avoidable by eliminating oxidation, butter fat was irradiated in air, nitrogen, carbon dioxide, and hydrogen at a depth of 0.08 cm. Two hours was selected as the time of exposure, this being sufficient to effect complete destruction of vitamin A as revealed in the previous experiments. The butter fat in these experiments was fed separately in small containers, the basal ration being available for ad libitum consumption. In one series, animals were given 80 mgm. butter fat daily, and in the other 320 mgm.

Table 6 shows the results obtained with both levels of intake. It is evident that irradiation in air as contrasted with irradiation in nitrogen, carbon dioxide, and hydrogen, was more destructive, but on the other hand, preparations made in this manner compared with untreated butter fat showed that destruction of vitamin A was not prevented. In these experiments we also kept a record of the growth response of the animals, which gave results in entire harmony with the cure of ophthalmia. In order to conserve space these data were not charted.

In our experiments we did not obtain any evidence of toxicity of irradiated butter fat as reported by Spinka (2). It is well known that with excessive irradiation not only does there result complete destruction of vitamin A, but also bleaching and transformation of butter fat to a tallowy substance. When the exposure is continued to such extremes, it is, of course, entirely possible that not only unpalatability, but substances inimical to the welfare of the animal are produced as well. Willimott and Wokes (14, 15) claimed that, in cod liver oil, destruction of vitamin A continued after irradiation. While this is a possibility in view of the fact that ultra violet treatment is known to produce ozonides, yet in our experiments we failed to find any demonstrable decrease in vitamin A with irradiated butter fat stored for a period of seven months (chart 5). The storage conditions selected were those of room temperature and ice box temperature. The butter fat used for these experiments was irradiated at a depth of 0.16 cm. and for 30 minutes under the Alpine Sun Lamp as previously described. These samples were fed in comparison with non-irradiated butter fat kept under the same conditions.

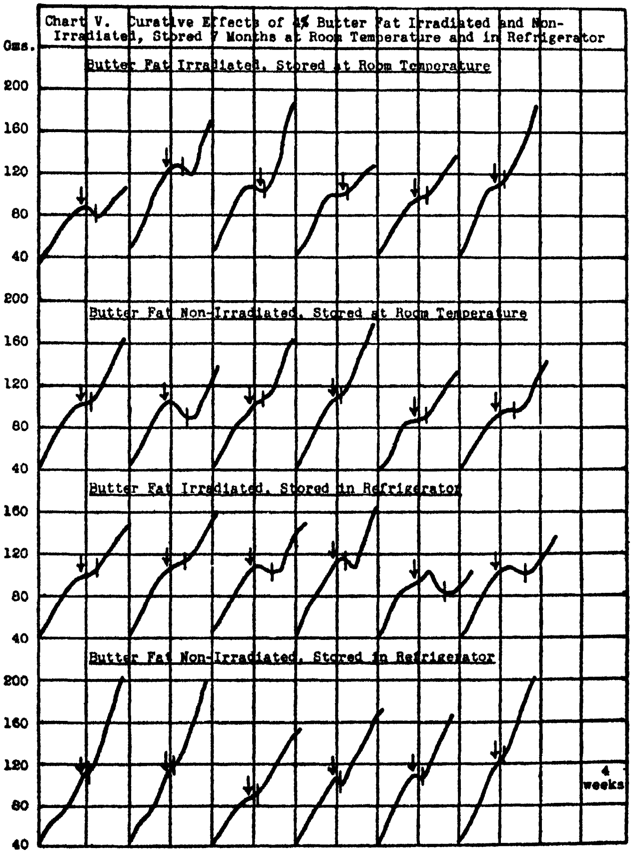


CHART 5

↓ incidence of ophthalmia, | change of ration, † death

Levels of intake were 1 and 4 per cent. To insure the animals getting a sufficient intake of the butter fat, records were kept throughout the experimental period.

Table 7 and chart 5, which present the data on ophthalmia and growth respectively, show that when stored at room temperature after irradiation for this comparatively long period, there had resulted little destruction of vitamin A. These changes must be interpreted in the light of the consumption records presented in table 8. In this table it is seen that the average consumption of some of the groups receiving the stored butter fat was slightly lower than the others. The difference in intake, however, was not of such an order as to be outside of experimental error, either due to variation in the groups of animals or accuracy of the experimental method when carried out under the best of conditions as recognized at the present time.

TABLE 7

Cure of ophthalmia with butter fat irradiated and non-irradiated and stored under various conditions 1 per cent in ration

WEEKS	IRRADIATED, STORED AT ROOM TEMPERATURE										NON-IRRADIATED, STORED AT ROOM TEMPERATURE						IRRADIATED, STORED IN ICEBOX							NON-IRRADIATED, STORED IN ICEBOX					
	Lot 74						Lot 81				Lot 76						Lot 78							Lot 80					
0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
1	3	2	2	3	3	1	3	3	3	2	0	0	0	1	2	2	0	2	2	0	1	2	2	3	3	0	1	1	0
2	1	†	0	3	2		3	3	1	0	0	0	0	0	1	0	0	0	2	0	1	†	0	0	3	0	0	0	0
3	1		0	3	0		†	2	1	0	0	0	0	0	1	0	0	0	2	0	1		0	0	0	0	0	0	0
4			0	0	0			1	†	0									0				0						
								†																					

4 per cent in ration

WEEKS	IRRADIATED, STORED AT ROOM TEMPERATURE						NON-IRRADIATED, STORED AT ROOM TEMPERATURE						IRRADIATED, STORED IN ICEBOX						NON-IRRADIATED, STORED IN ICEBOX								
	Lot 73						Lot 75						Lot 77						Lot 79						Lot 82		
0	3	3	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	3	4	3	4	3	3	3	3
1	0	1	2	0	0	0	0	3	2	0	0	0	1	0	1	2	2	1	2	1	2	2	2	1	0	1	0
2	0	0	1	0	0	0	0	3	1	0	0	0	1	0	0	1	0	1	1	0	1	0	1	0	0	0	0
3	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0

TABLE 8

Average daily consumption of individual animals during first two weeks or until death of animal died before being two weeks on supplemented ration.
(Butter fat stored seven months)

1 per cent in ration

	IRRADIATED, STORED AT ROOM TEMPERATURE										NON-IRRADIATED, STORED AT ROOM TEMPERATURE					
	Lot 74						Lot 81				Lot 76					
Grams consumed...	8 1	1 5	5 6	7 6	7 0	6 3	3 9	6 0	6 3	7.8	10.4	6 9	9 1	7 2	7 5	8.3
Average..... .	6 0										8 2					

	IRRADIATED, STORED IN ICEBOX							NON-IRRADIATED, STORED IN ICEBOX					
	Lot 78							Lot 80					
Grams consumed	7 8	6 0	6 4	8 5	6 5	4 7	6 1	8 2	8 2	9 1	9 9	6 1	7 3
Average . ..	6 6							8 1					

4 per cent in ration

	IRRADIATED, STORED AT ROOM TEMPERATURE						NON-IRRADIATED, STORED AT ROOM TEMPERATURE					
	Lot 73						Lot 75					
Grams consumed .	6.6	8 0	10 4	8 3	9 3	10 6	9 1	6 2	8 9	10 3	9 0	9 4
Average	8 9						8 8					

	IRRADIATED, STORED IN ICEBOX						NON-IRRADIATED, STORED IN ICEBOX								
	Lot 77						Lot 79						Lot 82		
Grams consumed..	8 4	8 5	7 8	7 8	4 6	6 9	11 5	10 6	8 6	9 3	9 4	12 0	11 4	9 9	13 6
Average..........	7 3						10 7								

SUMMARY

Different techniques for determination of vitamin A have been discussed. The Evans-Bishop technique was found to have no advantages over those techniques which depend upon the reinitiation of growth or the cure of ophthalmia. Of these latter the ophthalmic method was preferred, because of the specific nature of its reaction, and its rapid responsiveness. The growth method was also found reliable and correlated closely in results obtained with the ophthalmic method when the animals were taken for test shortly after growth ceased. Furthermore, with animals of variable weight we experienced difficulty in deciding just when they should be taken for the growth test.

Vitamin A is destroyed by ultraviolet treatment even when the irradiation is carried out in an atmosphere of CO_2, nitrogen, or hydrogen. However, these gases apparently do retard the destructive reaction somewhat. It is still possible that our preparations of butter fat may have contained a sufficiency of oxygen in solution to have allowed destruction by oxidation. This hypothesis appears tenable when one takes into consideration the very small amounts of vitamin A which can produce cure of ophthalmia or reinitiation of growth.

The destruction of vitamin A was found to be proportional to the time of exposure and inversely proportional to the thickness of the layer of fat exposed.

The irradiation necessary to secure maximum activation was found to cause some destruction of vitamin A. Practically, however, maximum activation may not be desirable from the dietetic standpoint, so that the antirachitic activation of butter fat by direct irradiation is a practical possibility without danger of destroying vitamin A.

Butter fat, antirachitically activated, was found to have retained most of its vitamin A even after storage for seven months at room temperature.

REFERENCES

(1) Zilva, S. S.: XIX. The action of ultraviolet rays on the accessory food factors. Biochem Jour., 1919, xiii, 164.

(2) Spinka, J.: Untersuchungen uber den Einfluss Ultravioletter Strahlen auf akzessorische Stoffe. Biochem Zeit., 1924, cliii, 197.

(3) Zilva, S. S.: Untersuchungen uber den Einfluss ultravioletter Strahlen auf die akzessorischen Nährstofffaktoren. Biochem. Zeit., 1925, clv, 333.

(4) Zilva, S. S.: LXXI. The action of ozone on the fat soluble factor in fats. Preliminary note. Biochem. Jour., 1920, xiv, 740.

(5) Peacock, P. R.: The action of light on cod liver oil. Lancet, 1926, ii, 328.

(6) Holmes, A. D., and Pigott, M. G.: Studies of the Vitamin potency of cod liver oils. XX. Effect of light on the vitamin A content of cod liver oil. Boston Med. and Surg. Jour., 1926, cxcv, 263.

(7) Titus, R. W., Hughes, J. S., Hinshaw, W. R., and Fitch, J. B.: Destruction of vitamin A in milk by ultraviolet light. Jour. Indus. and Engr. Chem. 1926, xviii, 843.

(8) Drummond, J. C.: The danger of irradiating milk. Jour. Amer. Med. Assoc., 1927, lxxxviii, 1014.

(9) Steenbock, H., and Coward, Katharine H.: Fat soluble vitamins. XXVII. The quantitative determination of vitamin A. Jour. Biol. Chem., 1927, lxxii, 765.

(10) Hogan, A. G., and Hunter, J. E.: The plural nature of vitamin B. Jour. Biol. Chem., 1928, lxxviii, 433.

(11) Supplee, G. C., and Dow, Odessa D.: Vitamin A potency of irradiated milk. Jour. Biol. Chem. 1927, lxxv, 227.

(12) Steenbock, H., and Black, A.: Fat soluble vitamins. XXIII. The induction of growth promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. Jour. Biol. Chem., 1925, lxiv, 263.

(13) Steenbock, H.: Calcium as a food and the relation of its assimilation to ultraviolet radiations. Physical Therapeutics, 1926, xliv, 272.

(14) Willimott, S. G., and Wokes, F.: Colour tests for vitamin A—their application to naturally occurring products. Lancet, 1927, ii, 8.

(15) Willimott, S. G., and Wokes, F.: The effect of irradiation on vitamin A. Pharm. Jour., 1927, cxviii, 217.

(16) Steenbock, H., and Wirick, Alice M.: Fat soluble vitamins. XXXI. Butter fat, its antirachitic properties and its artificial activation. Jour. Dairy Sci., 1930, xiii, 497.

(17) Steenbock, H., and Nelson, E. M. Fat soluble vitamins. XIII. Light in its relation to ophthalmia and growth. Jour. Biol. Chem., 1923, lvi, 355.

(18) Evans, H. M., and Bishop, K. S.: On the relations between fertility and nutrition. II. The ovulation rhythm in the rat on inadequate nutritional regimes. Jour. Med. Research, 1922, i, 335.

(19) Macy, Icie G., Outhouse, Julia, Long, M. Louisa, and Graham, Alice: Human milk studies. I. Technique employed in vitamin studies. Jour. Biol. Chem., 1927, lxxiii, 153.

THE RELATION OF CERTAIN PLANT PROCESSES TO FLAVOR DEVELOPMENT IN MARKET MILK*

P. H. TRACY AND H. A. RUEHE

Dairy Department, University of Illinois, Urbana, Illinois

There are various marketing operations necessary to give raw milk a desirable form and place utility. To determine the relationship existing between certain of these processes and the flavor development in market milk, this study was made.

MILK GRADING

To encourage the production of milk relatively free from bacteria many milk plants have adopted systems of grading, whereby the producers are rewarded or penalized according to certain bacterial standards. That buying milk on a bacterial grade basis will enable the plant operator to develop a raw milk supply of relatively low bacterial content is evidenced by the record of milk grading at the University of Illinois Creamery for the year ending September 1, 1928. During this period milk was purchased from forty to forty-five producers who averaged about 100 pounds each daily. A bacterial count was made on all milk once each week, using the Official Plate Method. A monthly summary of the record of the results is shown in table 1. The data show that 34.48 per cent of all deliveries investigated had a count not exceeding 50,000 bacteria per cubic centimeter, while only 34.92 per cent of all deliveries had a count of over 200,000 bacteria per cubic centimeter.

This record indicates that most of the milk delivered was relatively low in bacteria. However, as it was thought possible to make further improvements, a system of farm inspection was adopted. A bacterial count was made once a week (twice a week in summer) upon each patron's milk and in case the results indicated some neglect at the farm the University representative immediately called and conferred with the producer in regard to possible causes of the trouble.

---

* An abstract of a thesis submitted by P. H. Tracy in partial fulfillment of the requirement for the degree of Doctor of Philosophy in Dairy Husbandry in the graduate school of the University of Illinois, 1930. Received for publication October 10, 1930.

In addition, during June, July, and August, a temperature standard was established in order to encourage proper cooling of the milk at the farm. Any milk having a temperature of 70°F. or over when delivered at the Creamery was penalized one grade, the lowest of the four grades being excepted.

TABLE 1

Summary of bacterial counts on raw milk delivered to the university creamery

Percentage of all deliveries containing following bacterial counts

MONTH	1-10 THOUSAND BACTERIA PER CUBIC CENTIMETER		10-50 THOUSAND BACTERIA PER CUBIC CENTIMETER		50-200 THOUSAND BACTERIA PER CUBIC CENTIMETER		200-500 THOUSAND BACTERIA PER CUBIC CENTIMETER		OVER 500 THOUSAND BACTERIA PER CUBIC CENTIMETER	
	1927-1928	1928-1929	1927-1928	1928-1929	1927-1928	1928-1929	1927 1928	1928-1929	1927-1928	1928-1929
September	2 24	30 95	14 54	50 00	19 48	11 90	34 16	2 39	29 38	4 76
October	0 00	28 08	56 80	58 00	25 00	11 60	6 80	2 32	11 40	0 00
November	0 00	46 94	36 32	34 70	29 50	14 28	16 00	4 08	18 18	0 00
December	0 00	40 00	31 20	42 00	37 70	14 00	15 55	2 00	15 55	2 00
January	0 00	63 26	51 10	32 65	37 20	4 09	7 05	0 00	4 65	0 00
February	2 27	32 65	72 70	53 06	22 76	10 20	0 00	4 09	2 27	0 00
March	0 00	26 10	31.70	54 34	41 46	17 39	9 77	2 17	17 07	0 00
April	0 00	31 26	55 00	45 82	40 00	12 50	0 00	10 42	5 00	0 00
May	0 00	2 22	16 60	15 54	39 00	40 06	23 80	26 64	20 60	15 54
June	2 44	6 51	31.70	28 21	29 27	41.41	9 75	15 19	26 84	8 68
July	0 00	0 00	2.38	18 56	26 18	34 92	19 04	34 92	52 40	11 60
August	0 00	2 50	6 56	22 50	19 68	55 00	21 34	20 00	52 42	0 00
Average	0 58	25 87	33 88	37 95	30 60	22 28	13 60	10 35	21 31	3 55

A summary of the bacterial checks for the year beginning September 1, 1927, and ending August 31, 1929 is given in table 1. It is plainly evident that the grading program followed the second year was more effective in securing a raw milk supply of a low bacterial content than was that followed during the preceding twelve months. The average percentage of total deliveries having a count less than fifty thousand bacteria per cubic centimeter was increased from 34.48 to 63.82, while the percentage of total deliveries having a count over two hundred thousand was reduced from 34.92 to 13.90.

THE TEMPERATURE STANDARD

Before the development of the Methylene Blue Reduction test for bacteria in milk, it was often the practice to establish a temperature standard during the summer months in order to force producers to properly cool their milk. However, with the adoption of the bacterial standards the temperature factor in many cases was no longer considered. A study made during August in a milk plant handling about fifty thousand pounds of milk daily, the milk being graded on the Methylene Blue Reduction test, is of interest in this connection.

A summary of the data shows that of the lots of milk having a temperature of 70°F. or above when received at the plant, 39.4 per cent were Grade A, 45.4 per cent were Grade B, and 13.7 per cent were Grade C. Of those having a temperature below 70°F., 53.6 per cent were Grade A, 43.5 per cent were Grade B, and 2.9 per cent were Grade C. Seventy-six per cent of all the milk received had a temperature of 70°F. or over. Considering all the milk received, 70.1 per cent of the Grade A, 76.7 per cent of the Grade B, and 93.5 per cent of the Grade C milk had a temperature of 70°F. or over.

In a further study made at the University Creamery, it was noted that during the month of May, 1929, much of the raw milk was being delivered at a temperature over 70°F. During the last week in May the results of one day's temperature check showed that only three out of forty-four deliveries had a temperature below 70°F. The minimum temperature of the milk when received was 60°F., and the maximum temperature was 86°F. Since warm milk at the time of delivery is undesirable, the temperature standard referred to above was made effective June first. All milk having a temperature of 70°F. or over at the time of delivery (morning's milk included) was penalized one grade, the fourth or lowest grade being excepted. A summary of the data showing the relation between the milk grade and the temperature during June, July, and August is given in table 2.

It appears from these data that a temperature standard in conjunction with a bacterial standard will assist in improving the quality of milk through the encouragement of proper cooling. However, it will be noticed that the temperature of most of the milk as it reached the plant was such that, considering its age and possible bacterial seeding, bacterial growth would have been rapid had the milk not been immediately cooled or pasteurized.

For some reason farmers find it possible to produce milk of reasonably low bacterial content without paying much attention to cooling. Unpublished data by Prucha and Tracy show that this is possible when milk is handled in sterile utensils. Milk drawn from the udder of a cow directly into sterile glass tubes has been kept at 68°F. for fifteen hours without any appreciable increase in bacteria. It seems logical to conclude, therefore, that the low bacterial counts in the milks mentioned in the above studies were due to extensive use of chemical sterilizers on the part of the farmers, most of whom were known to use this method of utensil disinfection.

TABLE 2

Relation between milk grade and temperature

BACTERIA PER CUBIC CENTIMETER	NUMBER OF DELIVERIES	PERCENTAGE OF DELIVERIES	AVERAGE TEMPERATURE	MAXIMUM TEMPERATURE	MINIMUM TEMPERATURE	PERCENTAGE UNDER 70°F
			°F	*°F*	*°F.*	
Under 50,000	249	53.5	67.2	75	56	81
50,000-200,000	124	29.1	68.8	76	62	62
200,000-500,000	58	13.6	71.0	77	59	41.4
Over 500,000	16	3.7	69.4	80	56	37.5

It is evident that the milk grading program followed at the University Creamery has resulted in a raw milk supply of very low bacterial content. In turn it might be concluded that the quality of the milk was very high, since so much emphasis has been placed upon bacterial content that to some extent we have come to think of milk quality as being measurable by the amount of bacterial contamination. To be sure, only a small proportion of the milk received during the period of this study was of poor flavor due to bacterial action. Yet examination of a number of these lots of milk has shown that the official "yardstick," which specifies a bacterial standard only, is incomplete. Cowy, barny odors in the raw milk were common; puckery flavors were present in most samples; a few were tallowy, and some were metallic. On several occasions, milk was received which had a medicinal flavor.

PASTEURIZATION

The main purpose of pasteurization is to destroy bacteria present in milk. Incidently, however, the process results in the elimination of some of the undesirable flavors. Several separate deliveries of milk were pasteurized in glass bottles and cooled in the same container. The flavor of the raw milk varied considerably. It was noticed with the exception of some feed flavors that in practically all cases the barn flavors in the raw milk were partially or completely eliminated by pasteurization. Puckery flavors as well as those of a medicinal nature were not appreciably benefited by the heat treatment.

Continued holding of the milk at pasteurizing temperature should not be practiced because of the cooked flavor which results. In one experiment milk was placed in quart bottles which in turn were placed in a hot water bath in which the milk was heated to 142°F. At the end of every thirty minutes for a period of six hours a bottle was removed, and after cooling in cold water, it was placed in a 40°F. room. After twenty-four hours all the samples were judged for flavor. The samples removed at the end of thirty and sixty minutes were comparable. However, the third sample, the one held at 142°F. for ninety minutes had a cooked flavor. This cooked flavor defect became more noticeable in each successive sample, the one held six hours having a very pronounced cooked flavor. In batch pasteurization it is a good practice to drop the temperature of the milk in the vat to about 125°F. if the bottling will require much more than thirty minutes.

RELATION OF CERTAIN METALS TO FLAVOR IN MILK

Milk is usually exposed to metal surfaces from the time of its removal from the udder of the cow until it is placed in the glass or paper bottle in which it is marketed. Some studies have been made of the effect of dissolved metals upon the flavor of milk. In 1905 Golding and Feilman (1) found that milk passed over a partially detinned copper cooler acquired a metallic flavor in about eighteen hours. Later, various other investigators (2, 3, 4, 5) showed that the presence of certain metals in milk resulted in the development of a flavor defect described as tallowiness. In general, the metals that showed a corrosive action were the ones that were found to impart undesirable flavors to the milk products.

In order to study the relation of metals to flavor in milk, various metals were added in small quantities to milk before pasteurization. Milk was also pasteurized in the presence of small strips of different kinds of metals. Various flavor defects resulted, such as puckeryness, metallic flavors, and tallowiness, described by some writers as a pasteboard flavor. Iron, copper, German silver, and Benedict nickel were particularly harmful in this respect. Aluminum, tin, nickel and Allegheny metal had the least effect upon the milk flavor.

Lactic acid, caustic and sodium hypochlorite filtrates of tin, iron, lead, aluminum, copper, iron oxide, copper oxide, and aluminum oxide when added to milk affected its flavor. Of the metals studied, copper and iron were the most detrimental to milk flavor. Pasteurization of milk containing these filtrates was not found to be a factor in flavor development, nor was the fullness of the bottle of much significance.

RELATION OF DAIRY EQUIPMENT TO TALLOWY FLAVOR IN MILK

As has been shown, tallowiness is very likely to result from the presence of certain metals in milk. Tallowiness in dairy products is so called because of its resemblance to the flavor and odor of spoiled tallow. Holm and Greenbank (6) attribute the defect to an autoxidation of the butterfat, the reaction being hastened by such factors as heat, light, moisture, acidity, and enzyme action. These investigators also state that as a result of the oxidation the oleic acid radical is split, resulting in the formation of aldehydes and acids which possess the tallowy odor. Hunziker and Hosman (7), however, concluded "that talloviness is not the direct result of oxidized oleic acid, but rather is caused by products formed in the butter during the absorption of oxygen by the oleic acid, such as glycollic acid ester of oleic acid."

It seems from these studies that in the processing of market milk the one factor most likely to produce a tallowy flavor is the presence of certain metals, which may be added to the milk through its contact with utensils and equipment. Tin has been used commonly as a lining for milk cans, vats, coolers, and other equipment and is itself nearly harmless to the flavor of milk. Unfortunately the tinned surface does not always remain, and as it is gradually removed through wear, copper or iron surfaces are usually left exposed. As has been shown these two metals are very detrimental to milk flavor.

It is to be expected that any plant process which produces a corrosion of the metal linings of the different dairy utensils and equipment will indirectly contribute to a flavor defect of market milk. The results of Hunziker (8), et al., and Prucha, Tracy, and Tuckey (9) show that sodium hypochlorite solutions containing as little as 35 p.p.m. of chlorin and 0.5 per cent solutions of sodium hydroxide or trisodium phosphate will corrode all the metals ordinarily used in dairy equipment, with the exception of the polished chromium metals. It seems likely, therefore, that much of the raw milk entering the plant contains a certain amount of dissolved metal and that more is added in the handling of the milk in the plant. There would be an accumulative effect of the metal so that the development of a flavor defect, such as tallowiness, might be due to more than one source of metal contamination.

RELATION OF FAT CONTENT OF MILK TO TALLOWY FLAVOR

In order to study the relation of the relative percentage of fat in milk to the effect of added metal upon the flavor, samples of milk were prepared containing 4.9, 4, 3, 2, 1, and 0 per cent of butterfat. To each lot of milk a small quantity of a lactic acid solution of copper oxide was added. The samples were held at 40°F. for seventy-two hours before being judged. In the absence of fat the copper salt caused a metallic flavor, but with the fat present a tallowy flavor resulted. As the fat content of the milk was increased the tallowy flavor became more apparent.

CONTAMINATION OF MILK AT THE FARM WITH METALS

It is possible that by the time the raw milk arrives at the plant it will contain variable amounts of iron or copper salts. Small amounts of these metal salts probably could not be measured accurately, but by the addition of a definite quantity of a copper salt to equal portions of different deliveries, differences in the flavor developing might be explained through differences in the total amount of iron or copper salts present. Accordingly, several samples of raw milk were treated in this way. Each of the raw milks was tested for chlorin, using the method devised by Rupp (10), and the bacterial count of each was determined. After pasteurization in glass bottles the milk was divided into two parts. To one part a small quantity of a copper salt was added. The samples were all stored at 40°F. for forty-eight hours before being judged. The results showed a marked difference in the way in which milks produced by different farmers (proper allowance being made for variations in butterfat) were affected by the addition of a small amount of copper salt.

In several instances the control samples—those to which no copper had been added—had a noticeable tallowy flavor after being held for forty-eight hours at 40°F. The majority of the producers supplying the milk used in this experiment were using a sodium hypochlorite sterilizer at the time of the experiment (July). The tests for chlorin suggest that chemical sterilizers had been used for sterilizing some of the equipment at the farm. It seems reasonable to conclude that in some cases at least an appreciable amount of copper or iron salts gets into the raw milk through the farm utensils.

CONTAMINATION OF MILK AT THE PLANT WITH METALS

a. Relation of tinned-copper internal tubular cooler to tallowiness

The passage of milk through the plant is accomplished by means of glass and metal equipment. From the data and discussion already presented in regard to the effect of certain metals upon milk flavor, one would expect market milk quality to be impaired as a result of contamination by vats and other equipment made of metals which are unsuitable either because of their composition or because of the treatment they have received. Milk certainly should not be permitted to come into contact with either iron or copper. Plants using either strong alkali solutions or sodium hypochlorite mixtures on tinned equipment will eventually have trouble from tallowiness caused by the metals left exposed by the corrosion of the tin.

Such a situation was encountered at the University Creamery. An unclean, tallowy flavor was noticed in the pasteurized milk, especially during the winter months. At the bottle filler the milk flavor seemed satisfactory, yet numerous complaints were received from customers that the milk had a very undesirable flavor. On investigation, it was noticed that the customers making the complaints were being served from the milk cases that were the last to be removed from the refrigerated room. This milk was the first milk cooled each day. In order to determine whether there was a difference in the quality of the milk bottled first and that bottled last the following experiment was performed:

Three quarts each of the first bottled and last bottled milk from the first batch was saved on Tuesday, Thursday, and Saturday of each week for a period of six weeks. The samples were stored at 40°F. and were judged after two, four, and six days. The results are given in table 3.

The milk (usually about two hundred gallons) was pasteurized in a glass lined vat and forced by air through two sections of 1.5 inch internal tubular coolers 12 feet long with 12 tubes in each section. The construction of the tubes was tin on copper. Various types of chemical sterilizers had been used in disinfecting the coolers, and some copper was exposed. The coolers were washed and rinsed usually in the afternoon and were disinfected the following morning just before using, with a chlorin solution containing 50 to 75 p.p m. of chlorin.

In an experiment made as a preliminary to the one summarized

TABLE 3

Flavor development in first and last milk bottled—flavor criticism

AFTER 2 DAYS		AFTER 4 DAYS		AFTER 6 DAYS	
First bottled	Last bottled	First bottled	Last bottled	First bottled	Last bottled
Tallowy	Good	Tallowy	Slight tallowy	Very tallowy	Slight tallowy
Slight tallowy	Good	Slight tallowy	Good	Slight tallowy	Good
Very tallowy	Good	Very tallowy	Good	Very tallowy	Good
Very tallowy	Good	Very tallowy	Tallowy	Very tallowy	Tallowy
Tallowy	Good	Tallowy	Good	Tallowy	Good
Slight tallowy	Good	Slight tallowy	Good	Slight tallowy	Good
Very tallowy	Good	Very tallowy	Slight tallowy	Tallowy, puckery, bitter	Slight tallowy
Very tallowy, slight medicinal	Slight tallowy	Very tallowy	Tallowy	Very tallowy	Tallowy
Tallowy	Good	Tallowy	Good	Very tallowy	Slight tallowy
Tallowy	Good	Very tallowy	Slight tallowy	Very tallowy	Good
Very tallowy	Good	Very tallowy	Tallowy	Very tallowy	Tallowy
Slight tallowy	Good	Slight tallowy	Good	Tallowy	Good
Slight tallowy	Good	Slight tallowy	Good	Tallowy	Good
Tallowy	(No sample)	Tallowy	Slight tallowy	Very tallowy	Tallowy
Tallowy	(No sample)	Tallowy	(No sample)	Tallowy	(No sample)
Tallowy	(No sample)	Tallowy	(No sample)	Very tallowy	(No sample)
Slight tallowy	Good	Slight tallowy	Slight tallowy	Slight tallowy	Slight tallowy

in table 3, an attempt was made to avoid the development of the tallowy flavor by pumping a thousand pounds of rinse water through the coolers after disinfecting with the chemical sterilizer. This was apparently of no advantage. The use of the chemical sterilizer was then discontinued altogether, but the tallowy flavor still persisted.

The tallowy flavor described in these experiments seemed to be due primarily to the presence in the milk of dissolved metals, possibly copper oxide. Probably the first milk to pass through the coolers received most of the metal oxide, whereas the last to pass through was only slightly contaminated.

b. Benedict nickel internal tubular coolers as a source of off-flavors in market milk

The unsatisfactory results obtained with the tinned copper internal tubular coolers led to their replacement with tubes made from Benedict nickel, an alloy of nickel, copper, and zinc. Soon after the installation of this equipment a peculiar, unclean, tallowy flavor was noted in some of the milk twenty-four to forty-eight hours old. In order to locate the source of this flavor defect, the following experiments were made:

The mixed herd milk of the regular run (about 250 gallons) was used in this experiment. The milk was pumped through an internal tubular preheater, the inner tubes of which are made of Benedict nickel, into a vat lined with Alleghany metal. After pasteurization some of the milk was cooled in a well-tinned bucket submerged in cold water. Some of this milk was then transferred to a glass bottle. About two quarts of the hot milk were then pumped through the pump (a brass piston pump) regularly used to force the milk from the vat through the coolers. After cooling in a tin bucket, a quart of this milk was placed in a glass bottle. The remaining milk was then pumped through the cooler into the bottle filler, a sample of the first milk discharged at the end of the cooler being saved. The first and last bottles of milk bottled were also saved. The results are given in table 4.

It was evident that although the pump was a possible source of tallowy flavor,[1] the internal tubular coolers were a more important factor in contributing to the tallowy flavor in the milk. The flavor defect due to the use of internal tubular coolers was similar to that caused by the partially detinned copper tubes previously referred to, but the milk usually had an additional flavor described as unclean. Examination of a large number of samples of the first and last milk bottled clearly showed that the first milk through the coolers was of poorer quality than the last

TABLE 4

Comparison of samples taken at different points in the cooling process—flavor criticism

SAMPLE	A		
	After 7 hours	After 24 hours	After 48 hours
Directly from vat	Slight puckery	Slight puckery	Slight puckery
First through pump	Slight metallic	Slight tallowy	Slight tallowy
First through cooler	Watery, unclean sterilizer flavor	Tallowy, watery, unclean	Very, very tallowy
First bottle at filler	Fairly good	Tallowy	Tallowy
Last bottle at filler	Fairly good	Slight tallowy	Slight tallowy
	B		
	After 7 hours	After 24 hours	After 120 hours
Directly from vat	Good	Good	Old flavor
First through cooler*	Watery, unclean, sterilizer flavor	Watery, unclean, sterilizer flavor, puckery	Watery, puckery, very tallowy
First through pump	Slight metallic, puckery	Metallic, puckery	Metallic, tallowy
First bottle at filler	Fair to good	Slight tallowy	Tallowy
Last bottle at filler	Good	Slight tallowy	Tallowy

* A Mojonnier test on the milk from the vat showed a fat content of 4.06 per cent, that from the pump tested 3.85 per cent, while the first from the cooler tested 3.63 per cent. This dilution was in spite of efforts made to drain the system of rinse water.

---

[1] It is interesting to note that a small lot of milk (about 1 gallon) pasteurized in the presence of the removable parts of the Simplex brass pump developed a very strong tallowy flavor in twenty-four hours, whereas the control sample was of good quality.

milk cooled. There was a variation in the seriousness of the defect from day to day, yet the flavor would invariably develop in time. Peculiarly the trouble was much more noticeable in winter than in summer. The off-flavor would not develop until after the milk had been stored at 40°F. for twenty-four to forty-eight hours. Contrary to expectation the flavor developed faster in milk stored at 40°F. than that stored at 68°F. The flavor developed regardless of whether steam or chemical sterilizers were used on the equipment.

In order to obtain further evidence that the internal tubular coolers were the source of the off-flavor, the following experiment was performed over a period of several days:

The raw milk to be used for bottling was pumped directly from a well-tinned dump vat into the Allegheny steel vat. After pasteurizing at 142°F. for thirty minutes the milk was cooled in the following ways:

Cooling method number	
1	Dipped from vat and poured over surface cooler no. 1
2	Dipped from vat and poured over surface cooler no. 2
3	Dipped from vat and poured over surface cooler no. 3
4	Dipped from vat and poured over surface cooler no. 4
5	Pumped from vat over surface cooler no. 1
6	Pumped from vat over surface cooler no. 2
7	Pumped from vat over surface cooler no. 3
8	Pumped from vat over surface cooler no. 4
9	Pumped through water section of internal tubular coolers and then poured over surface cooler no. 2
10	Pumped through both sections of internal tubular coolers
11	Dipped into glass bottle and cooled in bottle
12	Control—pasteurized and cooled in bottle

In the case of cooling methods nos. 1 to 9, 10 gallons of the milk were used. In the case of method no. 10, almost 100 gallons of milk were used. In the case of methods 11 and 12, 1 quart of milk was used. In all cases the first bottles cooled were saved for judging. The samples were judged after twenty-four and forty-eight hours, and the study was continued for one week. The results of one day's run are recorded in table 5.

It was evident from the data that the flavor of the milk may be affected by surface as well as by internal tubular coolers. The latter, however, seemed to be a more serious cause of an unclean and very often tallowy flavor. To be sure, the milk cooled in glass was not always perfect in flavor, indicating metal contamination of the milk prior to its being placed in the vat, a condition made more serious, however, by the subsequent contamination from the milk pump and coolers.

TABLE 5

Relation of method of cooling to tallowy flavor—flavor criticism

COOLING METHOD NUMBER	AFTER 24 HOURS	AFTER 48 HOURS
1	Metallic, unclean	Tallowy
2	Puckery but clean	Puckery
3	Metallic, unclean	Metallic, unclean
4	Slight metallic	Slight metallic
5	Tallowy	Tallowy
6	Slight tallowy	Slight tallowy
7	Unclean, metallic, puckery	Unclean, metallic, puckery
8	Metallic, puckery	Metallic, puckery
9	Unclean, puckery	Unclean, tallowy, puckery
10	Very unclean, puckery	Tallowy, puckery, unclean
11	Slight puckery	Puckery, slight unclean
12	Slight puckery but best of all samples	Slight unclean

EFFECT OF SUNLIGHT UPON MILK FLAVOR

That light hastens the development of a tallowy flavor in butter fat has been known for some time (11). Hammer and Cordes (12), however, were the first to report a tallowy flavor in market milk due to sunlight. Skim milk when exposed to sunlight, according to these investigators, developed an abnormal flavor that was not talloweiness. By the use of brown milk bottles they were able to prevent the occurrence of either of these two defects.

Frazier (13) explains the action of light as a catalyst in the oxidation of milk fat. He also suggests that a metal catalyst would assist the light in hastening the oxidation of the butterfat.

Schulz (14) states that the deterioration of the taste produced by ultra violet irradiation of milk is caused by the action of hydrogen peroxide on the proteins. Tiede and Reyher (15) concluded that the action was due to the activity of ozone.

In order to further study the significance of diffuse light and sunlight in their relation to milk flavors, a series of experiments were performed exposing milk varying in composition and amount of metal contamination to light rays under variable conditions.

It was found that two flavor defects resulted from the exposure of milk to sunlight, one a tallowiness and the other a burnt flavor. When the milk was exposed to the diffuse light only a tallowy flavor developed. The formation of this flavor was hastened by the presence of metals. The burnt flavor tended to mask the tallowiness when present and seemed to be due to an action upon the milk serum rather than the fat, as the defect was more noticeable in skim milk than in whole milk. There was no direct relation between the burnt flavor formation and the presence of copper salts.

RELATION OF THE USE OF CHEMICAL STERILIZERS TO FLAVOR IN MARKET MILK

Dairy utensils and equipment are treated in various ways in order to destroy the bacteria present. For many years hot water and steam alone were used for this purpose. Recently, however, chemical sterilizers have been extensively employed in the disinfection of both farm and plant equipment. There are various types of chemical sterilizers, but the ones ordinarily used for dairy utensils are those containing chlorin, such as the hypochlorites of calcium and sodium and organic compounds such as sodium-sulphorichloramid, more commonly called chloramine-T.

Prucha (16) has made extensive studies of the application of chemical disinfection to the dairy industry and concludes that chlorin compounds are suitable sterilizers for utensils. He warns against treating the milk with chemical sterilizers in order to destroy bacteria.

The amount of information in the literature relative to the effect upon milk flavor of the use of chemical sterilizers in disinfecting dairy utensils is limited. A series of experiments were performed, therefore, to study this factor.

It was found that chemical sterilizers added either directly to the milk or through treatment of the bottle affected the flavor of milk. A puckery, burning sensation usually resulted. When stronger concentrations of the sterilizing solutions were used, a sickening, unclean flavor resulted. Whether the sodium hypochlorite was alkaline or slightly acid did not seem to be of any importance from the standpoint of the effect upon flavor.

It was also found that the addition of sodium hypochlorite to milk caused a more pronounced tallowiness to develop in the samples containing a sufficient amount of metal to produce tallowiness, indicating an additional effect of the disinfectant. It was also noted that in most cases in which a tallowy flavor developed in milk exposed to metal strips, the defect was more pronounced in the milk brought into contact with the strips previously treated with a sodium hypochlorite solution.

An occasional occurrence of a medicinal flavor has been noted in the raw milk received at the University Creamery. A few complaints were received that the pasteurized milk "tastes as if it had medicine in it." No information could be found in the literature as to the cause of this defect.

Numerous attempts to reproduce the medicinal flavor by the direct addition of the sterilizer failed to result in anything more than the defects noted above. A series of experiments was next performed in which the milk was exposed to glass and metal surfaces treated with chemical disinfectants.

It was found that the defect was due to bringing milk into contact with tinned utensils and equipment treated with a chemical sterilizer a short time before using. If the tinned surface was allowed to dry before adding the milk or if the equipment was used immediately after treating with the chlorin compound, the defect usually did not occur.

According to Buswell (17) in the chlorination of city water supplies the presence of phenol is intensified by the addition of chlorin. The phenol flavor is not the same as the medicinal flavor discussed previously. Recently, Mattick (18) studied the cause of phenol flavor in sterilized milk and concluded it was due to bacterial action. According to Bell (19), p-cresol may be derived from the bacterial degradation of tyrosine, which can yield either p-cresol or phenol.

It was found that the addition of sodium hypochlorite to milk containing a small quantity of phenol greatly intensified the flavor. Since small quantities of phenols may be absorbed by the milk either from the air or by direct contact with contaminated utensils, and since phenol may result from bacterial action, the addition of small quantities of a chlorin sterilizer may result in a noticeable phenol flavor in the market milk, which otherwise might have escaped detection.

SUMMARY

The relation between a number of plant processes and the quality of market milk as indicated by its flavor has been studied. In the absence of more suitable methods the flavor of the milk was determined organoleptically.

One of the most important of the plant processes bearing upon the quality of market milk is the use of an effective grading system. Such a system should provide for bacterial, flavor, and temperature control in the raw milk up until the time of delivery.

Pasteurization improves the flavor of most raw milk. Continued heating at 142°F. for over one hour, however, may result in a noticeable cooked flavor.

Milk should not be brought into contact with either iron or copper surfaces. Benedict nickel also may have a detrimental effect upon milk flavor. The development of a tallowy flavor in milk is closely associated with metal contamination. Glass and Allegheny metal have practically no ill effects on milk flavor.

Two kinds of flavor defects may occur in milk exposed to sunlight, one a tallowiness, and the other a burnt flavor. Diffuse light is an important factor in the development of tallowy flavor in milk, especially that containing an added copper salt.

The use of chlorin sterilizers on farm and plant equipment should be confined to those of a non-corrosive nature. In no case should the disinfectant be added directly to the milk, and all utensils and equipment treated with chlorin sterilizers should be rinsed with uncontaminated water before adding the milk.

Otherwise, puckery, unclean, or medicinal flavors may develop. The odor and flavor of phenol, when present in milk is greatly intensified by the presence of chlorin in the form of sodium hypochlorite.

REFERENCES

(1) Golding, J., and Feilman, E 1905 Taint in milk due to contamination by copper. Jour. Soc. Chem. Indust., xxiv, 1285–1286.

(2) Quam, G. N., and Solomon, E. I. 1927 Some more facts about metals. The Milk Dealer, xvi, no. 5, 61, 62.

(3) Okuyyama, Sobey 1927 The bottle cap not to blame. The Milk Dealer, xvi, no. 11, 45.

(4) Hunziker, O. F. 1923 Metals in the construction of dairy equipment. Proc. World's Dairy Cong., 1923, (2), 1189–1202.

(5) Hunziker, O. F., Cordes, W. A., and Nissen, B. H. 1929 Metals in dairy equipment. Metallic corrosion in milk products and its effect on flavor. Jour. Dairy Sci., xii, no. 2, 140–181.

(6) Holm, G. E , and Greenbank, G. R. 1923 The keeping quality of butterfat, with special reference to milk powder. Proc. World's Dairy Cong., 1923, (2), 1253-1265.

(7) Hunziker, O. F., and Hosman, D F. 1917 Tallowy butter, its causes and prevention. Journ. Dairy Sci , no. i, 4, 77–106.

(8) Hunziker, O. F., Cordes, W. A., and Nissen, B. H. 1929 Metals in dairy equipment. Jour. Dairy Sci., xii, no. 3, 252–284.

(9) Prucha, M. J., Tracy, P H., and Tuckey, S. L. Unpublished data of the University of Illinois.

(10) Rupp, Phillip 1922 The detection of hypochlorites and chloramines in milk and cream. United States Department of Agriculture Bulletin, No. 1114

(11) Hunziker, O. F. 1926 Condensed Milk and Milk Powder, 4th edition, p. 501.

(12) Hammer, B. W., and Cordes, W. A. 1920 A study of brown glass milk bottles. Research Bulletin, No. 64.

(13) Frazier, W. C. 1928 A defect in milk due to light. Jour. Dairy Sci., xi, no. 5, 375–379.

(14) Schultz, Ottokarl 1927 New method of activation. Preliminary report. Milchwirkschafth Forschung, iv, 37–40. (Chem. Abstracts, xxi, 3631.)

(15) Tiede, E., and Reyher, P. 1926 Ultra-violet irradiation of milk. Naturwissenschaften, xiv, 741. (Chem. Abstracts, xx, 3197.)

(16) Prucha, M. J. 1929 Chemical sterilization of dairy utensils. University of Illinois Agricultural Experiment Station Circular 332.

(17) Buswell, Arthur M. 1928 The Chemistry of Water and Sewage. Page 199.

(18) Mattick, A. T. R. 1930 Phenols in sterilized milk. Analyst, lv, 37, 38.

(19) Bell, W. H. 1921 A method for the detection of phenols produced by bacteria. Jour. Infect. Dis., xxix, 424–428.

CALCIUM AND PHOSPHORUS METABOLISM IN DAIRY COWS

IV. THE ASSIMILATION OF CALCIUM FED AS CALCIUM GLUCONATE*

WILLIAM A. TURNER, EDWARD A. KANE, WALTER S. HALE AND HERBERT G. WISEMAN

Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture, Beltsville, Maryland

INTRODUCTION

Recent interest in gluconic acid has brought about improved methods of production (1) and developed uses for its calcium salt, in which form it is chiefly obtained. The calcium salt, because of its solubility, lack of taste, and other valuable qualities, has been found advantageous for use in calcium therapy (2). For this reason it seemed of interest to determine whether a compound of this particular type (containing five hydroxyl-groups) could be readily assimilated from the alimentary tract and so prove a valuable source of supplementary calcium in the ration of lactating cows. Accordingly a balance experiment was undertaken to investigate this point.

EXPERIMENTAL

Two pure-bred, three-year old, Holstein cows, in their first lactation were placed in the metabolism stalls on November 30, 1929. Cow 212 had calved August 7, 1929, and was giving 21 kgm. of milk per day. Cow 213 had calved August 13, 1929, and was giving 19 kgm. of milk per day.

The cows were given a basal ration consisting of United States Grade No. 1 alfalfa hay and a grain mixture made up as follows: wheat bran, 30; corn meal, 40; soy bean meal, 20; linseed meal, 10; and sodium chloride, 1. This was fed in such amounts as the cows would clean up and in sufficient quantity to more than satisfy their nutritive requirements, according to the standards of Savage (3).

---

* Received for publication October 29, 1930.

The experiment was divided into four three-week periods. The basal ration supplemented with calcium gluconate (5 per cent of the grain) was given during the first three weeks of the experiment. (Feeding began three days previous to the collection of excreta.) The basal ration alone was given during the second three weeks. During the third three weeks calcium gluconate was

TABLE 1

Percentage composition of feeds

PERIOD	FEED	H_2O	Ca	P	N
December 6 to January 10	Alfalfa hay I	12.10	1.300	0.241	2.58
January 11 to February 11	Alfalfa hay II	11.91	1.345	0.246	2.61
February 12 to March 2	Alfalfa hay III	11.39	1.339	0.255	2.58
December 6 to 16	Grain 60 G I	11.40	0.580	0.737	3.14
December 17 to 27	Grain 60 G II	11.60	0.570	0.747	3.11
December 28 to January 8	Grain 60 I	11.20	0.149	0.753	3.22
January 9 to 17	Grain 60 II	11.10	0.157	0.751	3.30
January 18 to 30	Grain 60 G III	11.15	0.586	0.697	3.07
January 31 to February 7	Grain 60 G IV	12.53	0.458	0.717	3.18
February 8 to 19	Grain 60 L I	12.23	0.561	0.745	3.20
February 20 to March 2	Grain 60 L II	12.34	0.555	0.717	3.12

TABLE 2

Percentage composition of milk

WEEK	COW 212			COW 213		
	Ca	P	N	Ca	P	N
December 9 to 15	0.108	0.101	0.528	0.120	0.094	0.502
December 16 to 22	0.113	0.097	0.534	0.122	0.091	0.498
December 23 to 29	0.111	0.104	0.544	0.120	0.091	0.506
December 30 to January 5	0.105	0.106	0.550	0.114	0.089	0.484
January 6 to 12	0.114	0.102	0.547	0.117	0.096	0.500
January 13 to 19	0.108	0.104	0.531	0.110	0.095	0.490
January 20 to 26	0.114	0.105	0.522	0.119	0.092	0.505
January 27 to February 2	0.107	0.106	0.528	0.118	0.096	0.492
February 3 to 9	0.109	0.104	0.546	0.116	0.091	0.485
February 10 to 16	0.114	0.104	0.539	0.121	0.089	0.506
February 17 to 23	0.114	0.101	0.539	0.121	0.095	0.502
February 24 to March 2	0.114*	0.104*	0.540*	0.115	0.094	0.498

* Sample lost, arbitrary figures used.

TABLE 3

Weekly calcium balances

WEEK	COW 212					COW 213				
	Ca in urine and feces	Ca in milk	Ca in feed	Ca balance	Milk yield	Ca in urine and feces	Ca in milk	Ca in feed	Ca balance	Milk yield
Calcium gluconate supplement										
	grams	*grams*	*grams*	*grams*	*kgm.*	*grams*	*grams*	*grams*	*grams*	*kgm.*
December 9 to 15	991.4	161.0	1184.4	+32.0	149.8	829.9	160.4	1025.2	+34.9	133.7
December 16 to 22	1045.1	165.1	1180.8	−29.4	146.1	884.9	164.7	1021.7	−27.9	135.0
December 23 to 29	1065.3	158.5	1178.1	−45.7	142.8	817.6	161.0	967.1	−11.5	134.2
No supplement										
December 30 to January 5	781.2	148.8	912.9	−17.1	141.7	547.0	142.4	622.9	−66.5	124.9
January 6 to 12	771.6	158.8	857.0	−73.4	139.3	589.8	159.7	676.5	−73.0	136.5
January 13 to 19	765.2	156.5	905.5	−16.2	144.9	635.1	150.5	747.0	−38.6	136.8
Calcium gluconate supplement										
January 20 to 26	978.1	170.0	1114.4	−33.7	149.1	883.5	157.9	987.3	−54.1	132.7
January 27 to February 2	933.2	160.4	1076.5	−17.1	149.9	881.5	160.0	977.0	−64.5	135.6
February 3 to 9	1018.5	156.6	1123.1	−52.0	143.7	785.7	153.6	915.6	−23.7	132.4
Limestone supplement										
February 10 to 16	1123.7	171.5	1183.2	−112.0	150.4	872.0	154.2	971.8	−54.4	127.4
February 17 to 23	1023.3	169.2	1180.3	−12.2	148.4	804.0	153.8	969.3	+11.5	127.1
February 24 to March 2	1056.3	167.6*	1177.7	−46.2	147.0	836.2	151.7	966.9	−21.0	131.9

* Sample lost, arbitrary figure used.

again added to the ration, and during the fourth and last three weeks limestone, supplying an equivalent quantity of calcium, was substituted for the calcium gluconate in the grain mixture.

The supplemented ration was fed first, preceding the feeding of the basal ration unsupplemented, thus reversing the usual procedure. In this way any favorable effect of the supplement upon calcium assimilation was enabled to operate while the milk yield was at its maximum and the drain upon calcium resources greatest. According to the usual practice where the supplement follows a period on a basal ration, if the balances improve, as the

TABLE 4

Weekly phosphorus balances

WEEK	COW 212				COW 213			
	P in urine and feces	P in milk	P in feed	P balance	P in urine and feces	P in milk	P in feed	P balance
Calcium gluconate supplement								
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
December 9 to 15	408.2	151.3	616.1	+56.6	391.0	125.7	569.0	+52.3
December 16 to 22	467.9	141.7	619.7	+10.1	420.1	122.9	572.4	+29.4
December 23 to 29	476.6	148.5	622.4	−2.7	446.8	122.1	565.4	−3.5
No supplement								
December 30 to January 5	469.6	150.2	626.2	+6.4	423.9	111.2	489.7	−45.4
January 6 to 12	457.7	142.1	575.5	−24.3	425.4	131.0	531.0	−25.4
January 13 to 19	425.6	150.7	588.1	+11.8	438.1	130.0	541.1	−27.0
Calcium gluconate supplement								
January 20 to 26	365.4	156.6	533.5	+11.5	384.1	122.1	510.8	+ 4.6
January 27 to February 2	383.3	158.9	530.4	−11.8	435.4	130.2	512.4	−53.2
February 3 to 9	437.8	149.4	586.6	−0.6	406.5	120.5	522.0	−5.0
Limestone supplement								
February 10 to 16	471.8	156.4	606.3	−21.9	429.0	113.4	539.9	−2.5
February 17 to 23	441.7	149.9	604.3	+12.7	389.1	120.7	530.9	+21.1
February 24 to March 2	450.2	152.9*	592.2	−10.9	409.5	124.0	526.4	−7.1

* Sample lost, arbitrary figure used.

experiment proceeds and the milk yield declines, it is uncertain whether the improved balances are attributable to the effect of the declining milk yields or to the supplement administered.

The alfalfa hay was chopped and stored in sacks. Three batches of hay and 9 batches of grain were used during the experiment. The cows were fed hay and grain twice each day and milked three times a day. They were not pregnant and were not bred during the experiment. They were weighed on the first

TABLE 5

Weekly nitrogen balances

WEEK	COW 212				COW 213			
	N in urine and feces	N in milk	N in feed	N balance	N in urine and feces	N in milk	N in feed	N balance
Calcium gluconate supplement								
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
December 9 to 15	2570.4	790.9	3603.6	+242.3	2359.8	671.2	3231.9	+200.9
December 16 to 22	2711.3	780.2	3592.8	+101.3	2405.5	672.3	3221.6	+143.8
December 23 to 29	2687.4	776.8	3584.7	+120.5	2234.1	679.1	3110.6	+197.4
No supplement								
December 30 to January 5	2749.2	779.4	3654.0	+125.4	1884.5	604.5	2745.1	+256.1
January 6 to 12	2593.6	762.0	3386.8	+31.2	2262.5	682.5	2993.1	+48.1
January 13 to 19	2561.6	769.4	3509.6	+178.6	2305.9	670.3	3126.9	+150.7
Calcium gluconate supplement								
January 20 to 26	2382.6	778.3	3243.0	+82.1	2311.7	670.1	2998.1	+16.3
January 27 to February 2	2458.7	791.5	3200.3	−49.9	2355.5	667.2	3006.9	−15.8
February 3 to 9	2532.8	784.6	3558.7	+241.3	2070.1	642.1	3059.7	+347.5
Limestone supplement								
February 10 to 16	2719.2	810.7	3561.3	+31.4	2228.8	644.6	3063.6	+190.2
February 17 to 23	2584.4	799.9	3538.0	+153.7	2084.0	638.0	3043.4	+321.4
February 24 to March 2	2593.1	793.8*	3503.6	+116.7	2164.4	656.9	3011.4	+190.1

* Sample lost, arbitrary figure used.

three days of each month and the weights averaged. The average weights (kilograms) for the months, December, 1929, January, February and March, 1930 were, respectively for cow 212—487, 502, 510, 527; for cow 213—490, 486, 504, 517; showing a steady gain in weight throughout the experiment.

The Ca/P ratio in the feed ranged mostly between 1.75 and 2.00 in the supplemented periods and between 1.25 and 1.50 in the unsupplemented period.

Table 1 shows the percentage composition of the feeds used and table 2 the percentage content of calcium, phosphorus and nitrogen in the milks. Tables 3, 4 and 5 give the calcium, phosphorus, and nitrogen balance data.

DISCUSSION

The cows sustained their milk yields remarkably well, one cow giving more during the last three weeks of the experiment than during the first three. Whether this is to be attributed to an inherent characteristic of the animals, to the effect of the calcium supplements which they received, or to some other factor is not known. Sanders claims that a cow's milk yield is best sustained during the first lactation (4).

The blood-plasma calcium for the two cows 212 and 213 rose from 9.3 and 9.1 mgm. respectively before the experiment to 10.1 and 9.6 mgm. two weeks after the experiment began. The calcium level at the end of the experiment was 9.9 and 10.2 mgm. respectively. This may have some significance.

A casual inspection of the calcium balances shows that both cows retained calcium during the first week. This may be explained by the suddenly increased calcium intake with the introduction of calcium gluconate and the lag in excretion of the former low-calcium ration. Another factor is the somewhat depleted state of the cows which had been milking for nearly four months when the experiment started. This is particularly true of cow 213 which had received less than her nutritive requirements for three of the four months and which displayed a greater average calcium assimilation throughout the experiment.

The fourth and the seventh weeks, when changes in calcium intake were made, might be expected to show the effect of lag in excretion, but the effect does not seem to be apparent here. Probably the somewhat depleted condition of the cows is a more important factor in explaining the positive calcium balances in the first week than the effect of lag in excretion.

The calcium balances are only slightly less negative when calcium gluconate was given than when no supplement was given indicating little value for a calcium supplement and the results in the comparison of calcium gluconate and limestone as supplements are contradictory, indicating no advantage of either one over the other.

The milk contained more calcium during the periods when a supplement was given.

The strongly positive phosphorus balances in the first week indicate that the retention of calcium during this week has permitted the simultaneous retention of phosphorus to restore depleted bone tissue.

The phosphorus balances, like the calcium, are less negative when calcium gluconate was given than when no supplement was given. The phosphorus balances show the same contradictory results in the comparison of calcium gluconate and limestone supplements, indicating no superiority of one over the other.

The nitrogen balances indicate a greater retention of nitrogen by cow 213, which may be explained by the fact that she was slightly underfed previous to the experiment.

The response which a cow exhibits to any given change in ration is doubtless dependent largely on her state of nutrition. Thus in the present experiment cow 213 shows smaller losses of calcium and greater gains in nitrogen than cow 212. She even shows a retention of calcium while receiving the limestone supplement. Cow 213 was somewhat undernourished when the experiment began. This serves to emphasize the necessity for care in the standardization of animals for nutrition experiments.

CONCLUSION

A supplement of calcium gluconate was of little value in improving the calcium and phosphorus balances of cows giving 19 to 21 kgm. of milk daily.

A supplement of limestone gave inconclusive results.

We are indebted to Drs. H. T. Herrick and O. E. May of the Bureau of Chemistry and Soils for the calcium gluconate used in this experiment.

REFERENCES

(1) May, O. E., Herrick, H. T., Thom, C., and Church, M. B.: Jour. Biol. Chem., 1927, lxxv, 417.
Herrick, H. T., and May, O. E.: Jour. Biol. Chem., 1928, lxxvii, 185.
(2) Rothlin: Schweiz. Med. Wochen, 1927, lvii, 388.
Kottman: Schweiz. Med. Wochem., 1927, lvii, 409.
(3) Savage, E. S.: New York Cornell Agr. Sta Bul. 323, 1912, 59-123.
(4) Sanders, H. G: Jour Agric. Sci, 1930, xx, Part 2, 145.

THE GERMICIDAL ACTION OF RAW MILK UPON AN ORGANISM OF THE SWEET CURDLING TYPE*

HAROLD R. CURRAN

Research Laboratories, Bureau of Dairy Industry, Department of Agriculture, Washington, D. C.

The fact that fresh, raw milk exerts a bactericidal or antiseptic action upon many bacteria is well established. Investigators who have studied this phenomenon in its relation to the keeping quality of milk generally agree that the germicidal property is of academic interest only and without significant influence upon the general nature of the changes taking place in the milk. It is generally supposed that the destructive or inhibiting action is entirely too feeble and transitory to be of any practical importance.

In the course of a study upon the growth of bacteria in milk an active sweet curdler was encountered which proved to be unusually sensitive to the bactericidal action of raw milk. The restraining action of several samples of mixed milk was sufficiently strong and prolonged to suggest that this factor may, under certain conditions, play an important rôle in influencing the quality of the milk. The sweet curdling culture was isolated from milk about three months previous to this study; it was a rapidly growing Gram-positive sporulating rod, with optimum temperature about 37°C.

In a preliminary experiment with the sweet curdler the raw Holstein milk was found to be more susceptible to coagulation than the raw Jersey milk. The samples were taken from two cans of the mixed milk of each breed chosen at random from a supply of about 15 cans. The following day the experiment was repeated and the result was similar, the variation being only in the degree of difference in the coagulation time. These observations suggested the presence of a germicidal substance in the Jersey milk.

---

* Received for publication November 3, 1930.

The thermolabile nature of the germicidal substance in milk indicated a means of ascertaining the cause of the delayed coagulation in these experiments. One portion of the divided samples from each breed was heated in the Arnold sterilizer for fifteen minutes. This temperature is in excess of that required to completely inactivate the germicidal substance. The heated and unheated portions were then adjusted to a temperature of 37°C. after which each was inoculated with equal quantities of a broth culture of the sweet curdler. When this procedure was followed, the raw milks curdled in the same order and at about the same intervals as before, but the heated Jersey sample curdled shortly before the heated Holstein sample. This result was considered to be conclusive evidence of the presence of a germicidal substance in the Jersey milk.

In order to learn something of the extent to which the raw Jersey milk affected the growth of the sweet curdler the following experiment was carried out. Fifty cubic centimeter samples of the thoroughly mixed milk of each breed was divided into two equal portions and put into separate sterile flasks. One portion received the heat treatment already described, the other was held untreated. The heated and raw samples were then immersed in a warm water bath and held until the temperature of the milk reached 37°C. All the samples were then inoculated with a small drop of an eight-hour broth culture of the sweet curdler. After these were thoroughly agitated the microscopic count was determined, following which the flasks were placed in an incubator at 37°C. Observations for coagulation were made at hourly intervals and counts repeated at the end of four and eight hours. The direct microscopic method of enumeration was employed in preference to the plate method for the reason that consistent plate counts could not be obtained with the test organism. This difficulty is not infrequently encountered in plating aerobic spore formers. The direct microscopic count also served to minimize the error introduced into the plate method by the tendency of *Streptococcus lactis* to grow in pairs and chains. In most of this work the milk was five to six hours old at the time the samples were taken. Occasionally the samples represented a mixture of the morning's and previous night's milking.

Table 1 shows the results obtained in one of these experiments. After inoculation each sample contained about the same number of the test organisms. At the end of five hours the Holstein raw milk contained twice as many of these organisms as the Jersey raw sample. At the 9½-hour period the ratio was considerably reduced, indicating that the inhibiting agent was less effective. In this experiment, however, a slight difference was still manifest at the end of twenty-three hours. During the first nine hours the rate of growth in the inoculated raw and heated Holstein samples was quite similar, though the development was always somewhat greater in the latter. After twenty-four hours this difference was usually much greater, although this result was somewhat variable. Coagulation of the Jersey raw milk was delayed three and one-half hours in this experiment. The data would indicate that coagulation was not entirely dependent upon numbers, as the Holstein sample curdled with much fewer organisms than the corresponding Jersey sample. Differences in the salt content (buffer action) of the milk of the two breeds may have exerted some influence upon the coagulation time.

TABLE 1

Growth of the sweet curdler in raw and heated Holstein and Jersey milk, and time of coagulation

SAMPLE	BACTERIA PER CUBIC CENTIMETER				COAGULATION TIME
	0 hours	5 hours	9½ hours	23 hours	*hours*
Holstein, raw*	65,400	11,550,000	43,000,000	86,800,000	6
Holstein, heated	64,900	12,600,000	47,600,000	131,600,000	5¾
Jersey, raw†	64,100	5,250,000	40,810,000	82,000,000	9½
Jersey, heated	65,200	11,620,000	46,970,000	91,000,000	5½

* Plate count before inoculation 670.

† Plate count before inoculation 1030.

In table 2 are recorded the results of another experiment in which only raw milk was used. Divided samples from two cans were inoculated with the sweet curdler alone, and with a similar quantity of both the sweet curdler and *S. lactis*. Counts were made only upon the sweet curdler. At the end of three hours, both Holstein samples contained nearly four times as many organisms as the corresponding Jersey samples. At four hours the ratio was over 5 :1. In eight hours the inhibiting effect was less pronounced. The marked restraining action evident here is reflected in the relative coagulation times, the difference being over six hours. Under these conditions the sweet curdler was not appreciably affected by association with *S. lactis*.

TABLE 2

The sweet curdler grown independently and in combination with S. lactis in raw Holstein and Jersey milk, and time of coagulation

MILK	INOCULUM	NUMBER OF SWEET-CURDLING BACTERIA PER CUBIC CENTIMETER				COAGULATION TIME
		0 hours	3 hours	4 hours	8 hours	
						hours
Holstein	Sweet curdler	28,000	1,040,000	3,400,000	30,000,000	4½
Holstein	Sweet curdler and *S. lactis*	28,200	1,120,000	3,000,000	26,000,000	4½
Jersey	Sweet curdler	25,000	296,000	640,000	14,200,000	10¼
Jersey	Sweet curdler and *S. lactis*	26,100	226,000	620,000	15,200,000	10½

Holstein count before inoculation 5000.
Jersey count before inoculation 2100.

TABLE 3

The sweet curdler and S. lactis grown in combination in raw and heated Holstein and Jersey milk, and time of coagulation

SAMPLE	BACTERIA PER CUBIC CENTIMETER								COAGULATION TIME
	0 hours		4 hours		8 hours		22 hours		
	Sweet curdler	*S lactis*	Sweet curdler	*S lactis*	Sweet curdler	*S lactis*	Sweet curdler	*S. lactis*	
									hours
Holstein, raw*	29,000	448,000	8,300,000	21,790,000	19,880,000	27,160,000	94,500,000	939,900,000	4¾
Holstein, heated . . .	29,300	378,000	9,200,000	21,000,000	26,600,000	44,200,000	180,000,000	1,260,000,000	4½
Jersey, raw† .	33,000	350,000	968,000	19,148,000	8,820,000	31,000,000	121,800,000	327,600,000	9¾
Jersey, heated	30,000	500,000	9,960,000	22,260,000	26,600,000	22,820,000	242,200,000	1,026,900,000	4

* Plate count before inoculation 330.
† Plate count before inoculation 1700.

In table 3 another series of results is presented. In this experiment both the sweet curdler and *S. lactis* were inoculated into the raw and heated milks of both breeds. The pronounced restraining action of the raw Jersey milk upon the sweet curdler was evident after four hours, the ratio of the numbers of the sweet curdler in the Holstein and Jersey milks at this time was more than 9:1. In eight hours, though the ratio was considerably less, the Holstein milk still contained twice the number of test organisms present in the Jersey milk. At twenty-two hours a difference was still evident. The growth of the *S. lactis* in the raw milks was quite similar. There appeared to be a slight restraining action upon these organisms during the first four hours, but at the end of eight hours in this experiment there were slightly more *S. lactis* in the Jersey raw than in the Holstein raw. In general agreement with the results in table 1, the sweet curdler developed somewhat more rapidly in the heated milks during the first eight hours, but at twenty-four hours the difference was much greater. In this experiment there was a difference of five hours in the curdling time with raw milks. There appears to be somewhat better correlation between the number of organisms and the coagulation time here, as coagulation apparently occurred in both samples when the numbers were in the vicinity of 10 million. The coagulation of the heated milks was in the usual order and at about the same intervals.

DISCUSSION

Experimental evidence suggesting that the germicidal phenomena may be of general commercial importance in milk production is quite in harmony with some of our modern practices. Throughout the New York Milk Shed and in many other parts of the United States, morning's milk is never cooled before delivery to the milk plant. The night's milk is often cooled by submerging the warm milk in 10-gallon cans in ice water. Under these conditions the cooling takes place rather slowly. It has been shown by Marquardt and Dahlberg (1) that these practices are possible only because of the germicidal property of the milk.

It should be observed that the germicidal property of the milk in these studies was determined at 37°C., the optimum temperature for the test organism. Hunziker (2) and other investigators have shown that the germicidal phenomena is most evident at about 21°C., although the effect is of longer duration at still lower temperatures. In view of this fact it seems quite probable that a more pronounced effect would have been observed at lower incubation temperatures. The fact that the samples in this study were all taken from full 10-gallon cans of milk, representing the milk of from 3 to 6 cows, would suggest that the germicidal substance was present in relatively high concentrations in most of the Jersey milk for, if only present in the milk of a few cows, the effect would have been greatly reduced or lost by the high dilution. It is true that a restraining action, even of the intensity found in these milks, would under ordinary conditions have little influence in maintaining a low total bacterial count due to the fact that the usual predominating types of Micrococci, *S. lactis*, and others are only slightly affected. Nevertheless, when the milk supply is subject to heavy contamination with organisms of the sweet curdling type, and is subsequently held at relatively high temperatures for several hours, a strong germicidal action may have an important protective influence. This would be true especially during the hot summer months when temperature conditions for this type of organism are near the optimum and opportunity for contamination of all kinds is very great. When milk produced under these conditions is condensed, evaporated, or otherwise utilized within four to eight hours a strong bactericidal action may be instrumental in the prevention of heavy spoilage losses.

SUMMARY

Certain samples of mixed raw milk exerted a marked restraining action upon an organism capable of rapidly producing a sweet curdling type of fermentation. This action was present in the milk over a period of several months.

Coagulation produced by the sweet curdler was delayed from three and one-half to six hours in different samples of mixed raw milk.

Coagulation usually occurred when the sweet curdler attained a population of from 10 to 40 million.

The marked germicidal activity in this study was confined to Jersey milk.

Under the conditions of these experiments, *S. lactis* exerted but little influence upon the development of the sweet curdler and was only slightly restrained by the bactericidal activity of the milk.

Both *S. lactis* and the sweet curdler multiplied more rapidly in the heated milk than in the non-germicidal raw milk. The difference was relatively small during the first eight hours, but thereafter was usually large.

These observations indicate that under certain conditions a strong germicidal action may be of distinct value in protecting the milk supply.

REFERENCES

(1) Marquardt, J. C., and Dahlberg, A. C. 1930 Electric cooling of milk on the farm. N. Y. Agric. Exp. Sta. Bul. No. 581.

(2) Hunziker, O. F. 1901 Investigations concerning the germicidal action in cow's milk. Cornell University Agr. Exp. Sta. Bul. No. 197.

DEFICIENCIES IN RATIONS DEVOID OF ROUGHAGE FOR CALVES*

I. THE EFFECT OF THE ADDITION OF COD LIVER OIL AND ALFALFA ASH

S. W. MEAD AND W. M. REGAN

Division of Animal Husbandry, University of California, Davis, California

That the failure to secure continued normal growth in dairy animals reared on rations devoid of roughage results from a deficiency of Vitamin A alone or in conjunction with an inadequate supply of certain minerals, and not from a lack of roughage or bulk *per se*, is the conclusion to be drawn from the first of a series of experiments now being conducted by the Division of Animal Husbandry of the University of California at Davis.

Attempts have been made by a number of investigators to rear calves on milk alone or on milk plus some concentrate mixture designed to furnish all of the known requisites for their growth and well-being. Most of these experiments have failed; none has been entirely successful. The calves have either died at an early age or developed various deficiency symptoms and failed to make normal growth.

As early as 1897, Davenport (1) concluded that satisfactory growth and development could not be secured when calves were fed rations containing no roughage. Twenty-five years later these findings were corroborated by McCandlish (2), who states: "The lack of bulk may arrest the development of the alimentary tract and prevent the proper digestion of the nutrients supplied by the milk." Results of investigations by Huffman (3) indicate that the beneficial element in roughage must be something other than bulk of crude fiber. He states: "Coarse feeds in the form of corn cobs, oat hulls, and shavings, when added to concentrate rations have failed to prevent the onset of convulsions. Ap-

---

* **Received for publication November 11, 1930.**

parently, hay furnishes some factor other than bulk which is essential in the rations of cattle."

Although Bechdel, Eckles, and Palmer (4) succeeded in raising dairy heifers to freshening time on a ration containing no hay, they used beet pulp to furnish roughage. At eight months of age, their calves developed typical symptoms of Vitamin A deficiency; but when cod liver oil was added to the ration, normal growth and development were secured. After the second week of lactation, further serious difficulties arose. The animals refused to eat and lost weight rapidly. All remedial measures except the feeding of hay proved ineffective. These workers conclude: "That the difficulty is due to a mere lack of bulk or roughage in the ration, rather than to a mineral or vitamin deficiency, we are not in position to say. It appears that the trouble may be the same as that reported by Reed and Huffman (8), McCandlish (9), and other investigators (10)."

In an attempt to grow calves without roughage a series of experiments was initiated, the first of which is described in t[illegible] paper.

The animals used in this investigation were males dropped in the University herd. Calves 23E, 26E, 28E, 30E, and 31E were pure-bred Jerseys; calves 27E, 29E, and 32E were pure-bred Holsteins. They were placed on experiment at two days of age and with one exception were muzzled at this time. No. 23E did not have a muzzle until he was a month old.

All calves received whole milk from birth to six months of age. They received in addition a concentrate mixture, fed *ad libitum*, which consisted of the following feeds: 400 pounds of rolled barley, 300 pounds of rolled oats, 300 pounds of wheat bran, 100 pounds of linseed meal, and 8 pounds of salt. In place of roughage, calves 26E, 27E, 28E, 29E, 30E, and 31E received an amount of alfalfa ash equal to that supplied by a normal alfalfa hay ration. Data obtained from a number of feeding trials where calves received a normal ration were used in calculating the amount of alfalfa ash to feed.

The ash was obtained by burning alfalfa hay in iron drums with wire screen covers, which prevented the escape of a large part of the finer particles. Foreign material was then removed by sifting.

Until the calves were six months old, the ash was reburned in an electric muffle The daily allotment, as calculated for each calf, was placed in individual containers. For the first six months it was administered orally by hand; subsequently it was mixed with the concentrates at each feeding.

Calf 23E received milk to six months, as did the rest of the calves, but only concentrates thereafter; while 32E was allowed, in addition to the concentrates, copper sulphate and ferric oxide in amounts sufficient to prevent nutritional anemia, as calculated from the requirements of small animals (5).

An accurate account was kept of the amount of feed eaten by each calf. In order to determine the rate of growth, body weight, heights at withers, and heart girth measurements were taken at intervals.

The responses noted in the calves are presented in table 1; a record of growth, as indicated by body weight, is given in table 2. Figure 2 shows graphically the growth of each calf as compared with Eckles' normal (6). As will be noted, the calves grew normally until they were between seven and nine months of age, when all showed signs of physical deterioration, indicated by impaired vision, marked loss of appetite followed by a decline in body weight, stiffness of the joints, and, in some cases, loss of muscular control. Certain calves were eventually unable to stand, and all scoured profusely at times. When these symptoms, which were apparently those of a Vitamin A deficiency, became acute, "Patches" tested cod liver oil was administered in doses of 25 cc. daily. As the animals grew older it was deemed advisable to increase the dose to 75 cc. The clinical records of the individual calves follow:

Calf 23E. This calf was always below normal in body weight after 240 days of age. His eyes began to water profusely at 229 days; a white spot appeared upon each cornea, and complete loss of sight ensued This condition was accompanied by rapid respiration and a profuse nasal discharge. The appetite completely failed, and the animal became very much emaciated; its joints became quite stiff. Beginning at 289 days of age, cod liver oil was administered. Within one week there was a marked improvement in appetite, the eyes stopped watering, and the sight became apparently normal. At 523 days of age the daily administration of cod liver oil was increased to 75 cc.

Calf 26E. The clinical picture was much the same as that of 23E, except that about three weeks were required for eyesight to become normal after the first dose of cod liver oil was given at 256 days. That the breeding powers of this bull were not affected at 394 days of age is proved by the fact that a heifer bred to him became pregnant. At 490 days of age the cod liver oil dosage was increased to 75 cc. daily.

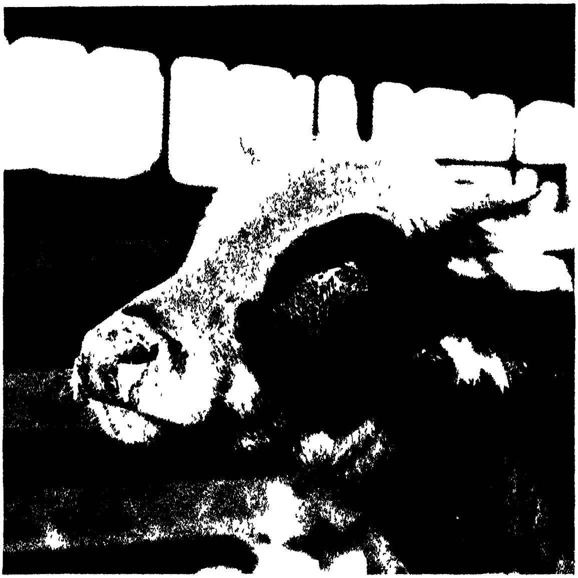


FIG. 1. CALF 29E

This condition, which developed in most of the calves at seven to nine months of age, was relieved immediately by the administration of cod liver oil.

Calf 27E. This was the only animal to die during the experiment. Post mortem examination showed the immediate cause of death to be a severe case of pleurisy. From 60 days of age, this calf made better than normal growth and showed no loss of appetite until ten days before death. He developed eye trouble at 232 days of age, the right eye suddenly turning white. His joints became stiff at 245 days of age. He was given cod liver oil at 257 days of age but died the next day.

Calf 28E. At 252 days of age, this animal began to exhibit a marked decline in appetite, and at 255 days, a loss of muscular control and impaired vision. No white spots appeared in the eyes, but at 291 days of age, he was in such poor physical condition that he was given cod liver oil. A gain in appetite was evident in 2 days; within a week his physical condition was greatly improved, and his eyesight was restored. The cod liver oil dosage was increased to 75 cc. daily at 450 days of age.

Calf 29E. Though there was no noticeable decline in appetite until 254 days of age, loss of muscular control and impaired vision were evident by the 248th day, and convulsions occurred on the 270th and 271st days. At this time he was totally blind; the left eye was matterated and white, badly ulcerated, and blood-shot. As shown in figure 1, the eyes were closed most of the time. Cod liver oil was administered on the 272nd day. Within 7 days, sight was restored to the right eye, which appeared normal. In another week there was considerable improvement in appetite and general vitality. By the time this calf was 292 days of age, vision had been completely restored, though a small white spot remained in the left eye. The rate of growth was also greatly accelerated. When 314 days old, however, he again began to evidence decline in appetite, to fall off in body weight, and to exhibit eye trouble. His daily dosage of cod liver oil was increased to 75 cc. He refused all feed for 15 days. In spite of the fact that an effort was made to stimulate his appetite by giving 6 to 8 pounds of whole milk daily, he lost 141 pounds in 30 days. During the next 10 days, he regained 54 pounds, and at 360 days of age he was eating well and rapidly gaining in weight.

Calf 30E. This animal showed decline in appetite at 230 days of age, though there was no noticeable loss in muscular control or stiffness of the joints. At 263 days, he had entirely lost his vision; and his watering and matterated eyes were closed most of the time. Cod liver oil in the usual amount was administered at 267 days. Within 2 days he exhibited a decided improvement in general health; the left eye was open and appeared clear and bright, though the right eye was still affected. By the time the calf was 276 days old, sight had been restored to both eyes; and he was eating well and gaining rapidly in weight. At 426 days of age the daily amount of cod liver oil was increased to 75 cc.

Calf 31E. Decline in appetite was evident at 226 days of age, and muscular control was gone by the 258th day. When 276 days old, he was totally blind. Two days later, cod liver oil was administered. Within a week his vitality had improved, and he had regained control of his movements; but he remained blind. At 414 days of age the cod liver oil dosage was increased to 75 cc. daily.

Calf 32E. This animal received copper and iron in place of alfalfa ash. In attempting to enter his stall at 216 days of age, he became jammed in the gate, injuring his hip. Though for a few days he was only slightly lame, the condition became accentuated, and after a month he remained in a prone position most of the time. His appetite began to fail at 222 days, and his eyes watered, though his vision at no time seemed to be badly impaired. Greatly emaciated, he appeared to be on the verge of death at 245 days of age, when cod liver oil was administered in the usual amounts. Improvement was slow; but by the 268th day his appetite had improved, and by the 290th, he was able to walk, though his left hip showed evidences of being broken. He gained in weight rapidly to 360 days of age, when the lameness was again accentuated and loss in weight occurred. Later he regained some of the loss but was still below normal in body weight. At 402 days of age his cod liver oil dosage was increased to 75 cc. daily. His rate of growth increased so that at 500 days of age he was slightly above the normal in body weight.

It is appreciated that the number of animals used in this experiment is small. Complete individual records, however, were taken; and, as shown in the data presented, almost identical results were obtained. All calves developed the deficiency symptoms at approximately the same age and in a strikingly regular sequence. When the remedial measure was taken, the steps in recovery were made in all cases in the same order and at uniform intervals.

As far as the authors have been able to ascertain from a search of the literature, this is the first time that a group of calves has been reared to the age of 19 months on a ration devoid of roughage, and normal growth secured. While other investigators have indicated that bulk is unnecessary, no one has proved it so by successfully growing the calves without it. Although others have used with good results a ration containing no hay, they supplied roughage in the form of beet pulp. The experiment reported here has demonstrated that calves can be grown to at least 19 months of age on a ration devoid of roughage. As shown by figure 2, five calves, except for short periods when they were suffering from Vitamin A deficiency, were above normal weight.

That the concentrates were inadequate in Vitamin A is evident from the fact that all calves developed marked symptoms of this deficiency, which disappeared upon the administration of cod liver oil. But that Vitamin A is not the only essential which the concentrates failed to supply in adequate amounts is indicated by the fact that calves 23E and 32E, which received no ash, were considerably underweight during most of the experiment.

TABLE 1

Deficiency symptoms and responses to cod liver oil administration observed at ages indicated

CALF NUMBER	STIFFNESS AND LOSS OF MUSCULAR CONTROL	MARKED LOSS OF APPETITE	SIGHT AFFECTED	COD LIVER OIL ADMINISTERED	FIRST RESPONSE	SIGHT NORMAL
	age in days	*age in days*	*age in days*	*age in days*	*age in days*	*age in days*
23E		240	229	289	296	296
26E	Stiffness, 210	235	225	256	263	296
27E	Stiffness, 245	248	232	257	Dead at 258	
28E	Loss of muscular control, 255	252	255	291	293	297
29E	Loss of muscular control, 248	254	248	272	279	292
30E		230	263	267	269	276
31E	Loss of muscular control, 258	226	262	278	Increased vitality, 285 Complete muscular control, 313	
32E	Injured hip, 216	222	222	245	Appetite improved, 268 Able to walk, 290	

CONCLUSION

Calves can be reared to 19 months of age and normal growth secured on a ration containing no roughage, providing cod liver oil and alfalfa ash are supplied in sufficient amounts.

TABLE 2

Body weights

AGE	CALF 23E	CALF 26E	CALF 27E	CALF 28E	CALF 29E	CALF 30E	CALF 31E	CALF 32E
days	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Birth	46	54	87	49	90	57	55	102
30		52	98	80	115	62	59	96
60	73	67	162	105	160	114	112	157
90	105	123	203	165	233	182	182	240
120	172	160	290	250	321	220	200	282
150	212	235	398	285	390	290	245	372
180	295	330	450	341	484	355	270	420
210	350	370	544	400	450	400	335	486
240	337	409	575	393	575	397	332	440
260						353	350	403
270	324	380		360	561	381	355	397
280						390	367	
290				343	584	408	383	
300	311	438		374	599	421	393	
310				384	620	429	425	428
320				397	560	461	442	449
330	298	522		408	535	463	464	460
340				422	479	485	467	480
350				457	533	482	483	500
360	360	518		463	561	493	508	518
370	370	540		469	597	499	517	495
380	356	559		491	601	509	531	504
390	377	584		510	610	513	537	516
400	373	583		518	643	533	559	527
410	392	614		525	693	518	544	523
420	382	609		550	688	517	533	523
430	392	643		566	722	504	529	546
440	383	637		551	702			
450	380	640		541	698			
460	409	639		541	721	530	575	580
470	407	665						
480	409	661						
490	436	683		610	880	584	622	634
500	448	686						
510	457	667						
520	446	695		610	948	660	665	725
530	446						673	
540	451					703		
550		675		635	1035			
570	485			662	1080			
580		707						
600	435							
610		775						
620	500	774						

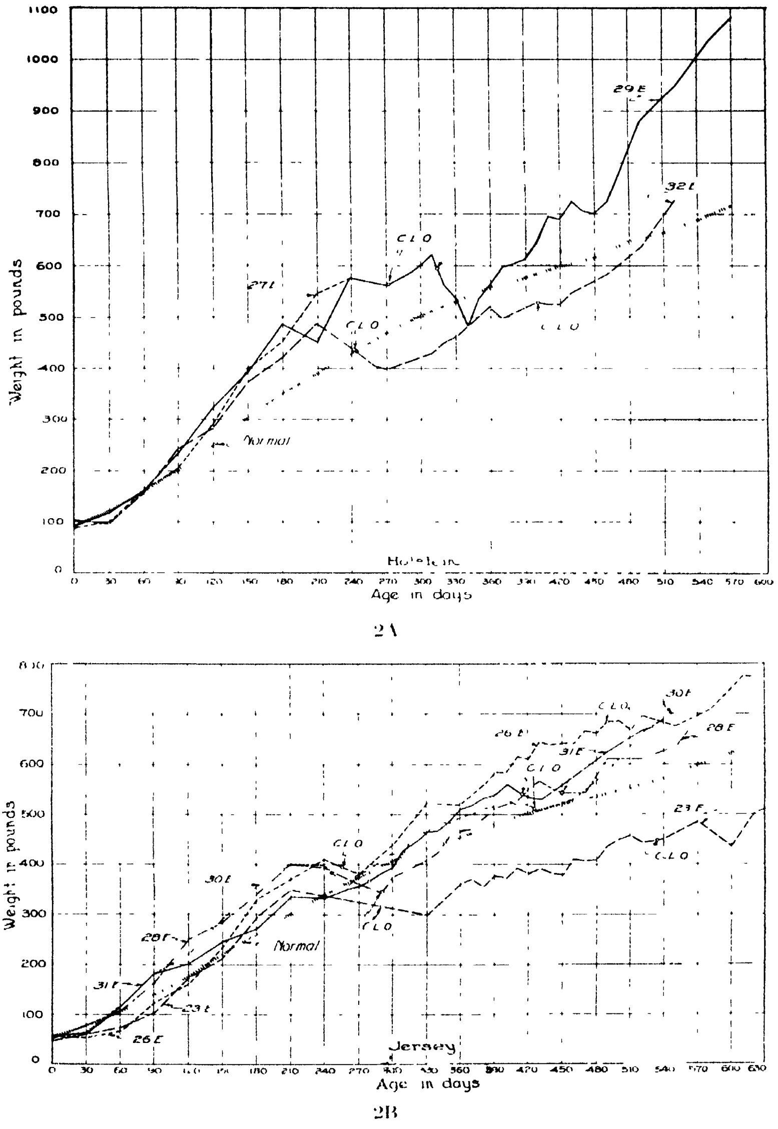


Fig 2 Individual Records of Growth as Measured by Body Weight Compared with Eckles' Normal

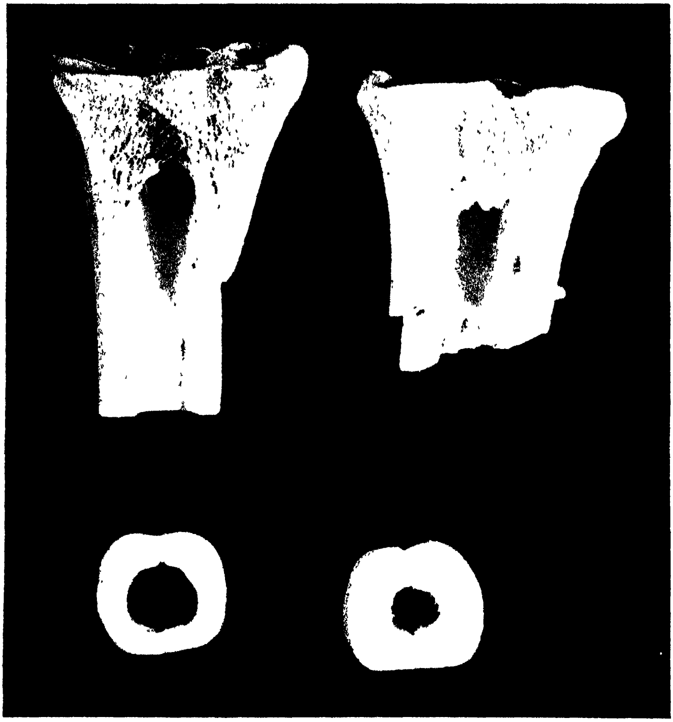
Calf 23E received milk and concentrates 32E received, in addition, copper sulphate and ferric oxide; all others, milk, concentrates, and alfalfa ash

TABLE 3

Analyses of canon bones of representative animals

ANIMAL NUMBER	WHEN SLAUGHTERED		DRY FAT-FREE BONE		MINERAL SUPPLEMENT
	Age	Body weight	Weight	Ash	
	days	*pounds*	*grams*	*per cent*	
23E (J)*	758	665	203	59.8	None
32E (H)†	647	925	284	53.8	None
26E (J)	725	904	264	66.5	Alfalfa ash
29E (H)	677	1,335	473	62.4	Alfalfa ash

* J Jersey
† H- Holstein



No. 23 E　　No. 26 E

FIG. 3 THE DEPOSITION OF MINERALS IN THE CANON BONES OF ANIMALS ON A ROUGHAGE-FREE RATION

No 23E received no mineral supplement, while No 26E received alfalfa ash.

ADDENDA

Because of limited barn facilities and the difficulties encountered in the management of two-year old bulls, the experimental animals reported upon above were slaughtered after the original manuscript had been submitted for publication. Data taken at the time of slaughter was, in the opinion of the authors, of sufficient importance to merit publication as addenda.

The following conditions were noted: A low deposition of minerals in the bones of 23E and 32E was indicated by the fact that their spinal columns could be readily cut with a knife, and that the canon bone of one was crushed while hoisting the carcas to the track. This was verified by the analyses of the left hind canon bones of representative animals as shown in table 3 and by figure 3. It is interesting to note also that the animals making the greater gain in each group showed the lower percentage of ash in the dry fat-free bone. The caul fat of all the animals appeared pure white. While they lacked the usual fill, the digestive tracts were otherwise normal. The dressing percentages were unusually high, averaging 57.4.

REFERENCES

(1) Davenport, E. 1897 On the importance of the physiological requirements of the animal body; results of an attempt to grow cattle without coarse feed. University of Ill. Agr. Exp. Sta. Bul. 46, 362.

(2) McCandlish, Andrew C. 1923 Studies in the growth and nutrition of dairy calves. V. Milk as the sole ration for calves. Jour. Dairy Sci., vi, 54.

(3) Huffman, C. F. 1928 Hay is necessary in ration of dairy cattle; unknown factor present in hay needed to maintain health of dairy cattle Quarterly Bulletin, Mich. Exp. Sta., xi, No. 1, 3.

(4) Bechdel, S. I., Eckles, C. H., and Palmer, L. S. 1926 The vitamin B requirement of the calf. Jour. Dairy Sci., ix, 409.

(5) Hart, E. B., Steenbach, H., Waddell, J., and Elvehjem, C. A. 1928 Iron in nutrition. VII. Copper as a supplement to iron for hemoglobin building in the rat. Jour. Biol. Chem., lxxvii, No. 2, 797.

(6) Eckles, C. H. 1920 The normal growth of dairy cattle. Mo. Agr. Exp. Sta. Research Bul. 36, 1–20.

LENGTH OF CALVING INTERVAL AND AVERAGE MILK YIELD*

W. L. GAINES AND J. R. PALFREY

Department of Dairy Husbandry, University of Illinois

The term "calving interval" refers to the period from one calving to the next calving. In dairy practice the most common calving interval is in the neighborhood of one year. Two somewhat different considerations may be responsible for this practice. There may be an economic advantage in having the flush of milk production, associated with calving, occur at some particular time of the year. There may be a biological advantage in having reproduction occur with a certain frequency. This paper is concerned with the latter consideration.

It is well known that delayed breeding and a consequent long calving interval is conducive to a large 365-day record, and the system of advanced registry testing has consequently favored such a practice. On the other hand, it is generally held that shorter calving intervals favor a large yield for the lifetime of the cow.

That frequent calving should give maximum lifetime yield seems in harmony with the known facts of the physiology of reproduction and lactation. The natural need of milk develops with birth of the young. The need increases with growth of the young so long as milk is the only food. As other food is taken the need for milk decreases and finally disappears. In milch animals, where we have adequate information as to the amount of milk produced day by day, it is found that the rate of milk secretion parallels roughly the rising and declining needs of the young. That is, the rate of milk secretion is most rapid in the cow shortly after calving and slows up quite markedly and continuously thereafter. It would seem that frequent calving by giving more high points in the lifetime lactation curve, should result in a greater total yield, as compared with less frequent calving.

Another way of putting the matter is to relate the length of the

---

* Received for publication November 19, 1930.

calving interval to the average yield of milk per day over the calving interval. This relation may be derived in relative terms from the known characteristics of the lactation curve. The indication is that the highest lifetime yield should be obtained from calving intervals of less than a year rather than from calving intervals of more than a year (1).

Matson (2) has recently pointed out an aspect of the problem which had not been considered before, namely, the effect of the calving interval on the *following* lactation. He states that while a short calving interval may go with high average yield for the current lactation, "it is paid for in the next lactation."

In view of the large expenditure of energy involved in the heavy milk secretion of improved dairy cows the point seems to be well taken. In the present paper the writers have thought, therefore, to utilize certain published data by way of further test of Matson's point.

MATERIAL AND TREATMENT

Langmack (3) has published the lifetime records of 425 cows, including for each cow and each calving interval, the length of the calving interval in days, the total milk yield for the lactation in kilograms, and the fat percentage of the entire milk for the lactation. The material seems to be suitable to cast light on the effect of the length of calving interval with respect to both the current and the *following* lactations. Furthermore, by the method of partial correlation the data may be treated to show the effect of calving interval on current yield with preceding calving interval constant; and on the following yield with the accompanying calving interval constant.

A few symbols will aid in presentation:

I = calving interval in days.
I_1 = first I; I_2 = second I; etc.
Y = average yield per day over the current calving interval, pounds of 4 per cent milk.
Y_1 = Y for I_1; Y_2 = Y for I_2; etc.
f = lactation fat percentage.
f_1 = f for I_1; f_2 = f for I_2; etc.

We may then calculate $r_{I_1Y_1}$, $r_{I_1Y_2}$, $r_{I_2Y_2 \cdot I_1}$, $r_{I_1Y_2 \cdot I_2}$, and so on through the succeeding lactations. Finally, we may calculate $r_{I_nY_n}$ and $r_{I_nY_{n+1}}$, where n takes successively any or all values within the limits of the data. This sort of treatment should bring out the relations in question so far as these particular data go.

After some working with the problem it appeared desirable to select certain of the records to carry through. Most of the records (352) are for cows of the Red Danish breed; the other records are for cows of the Ayrshire, Holstein, and Jersey breeds. It is desirable to work within the same breed. For each cow the records give ten or more lactations, but the tenth lactation is often incomplete. Some of the records do not start with the first calf. Body growth in general and mammary growth in particular, are factors which are most active during the first calving intervals. Finally, there is a good deal of abortion. It is plain that abortion will tend to raise the yield per day for the interval which it terminates, and decrease the yield per day for the interval which it begins. It is especially desirable to stay clear of the effect of abortion.

There are 186 cows of the Red Danish breed which have records starting with the cow's first calf and covering the first 9 calving intervals, all ten calvings being normal, that is, no abortion. These records constitute a unique body of data adapted to solution of the present problem, and were selected for detailed study, with the results presented in this paper. The selection reduces the number of records, which is not desirable. On the other hand the selection should give a very comparable succession of lactations, with many variables automatically eliminated, and this advantage is regarded as more than offsetting the sacrifice of numbers.

It seems to be a sound principle to measure lactation by energy yield and fat percentage, the one as a measure of the amount of work done, the other as an index of the kind of work done (4). The fat percentage is given directly in the records. Energy yield is computed in terms of 4 per cent milk by the formula, $FCM = M(0.882 + 0.331f)$ in which FCM is 4 per cent milk in pounds, M is milk in kilograms, and f is fat percentage. This computation together with the division for yield per day has been carried out by use of a 20-inch slide rule with an added fat percentage scale to fit the job. The conversion to pounds is made (with apologies) for the convenience of our readers who are mostly accustomed to thinking in terms of the English System.

RESULTS

The 186 cows under study were distributed among 13 different herds. In table 1 these herds are arranged in order of increasing calving interval to show the average yield per day over the first nine calving intervals, and the average weight per cow. The weights are given for only 155 of the 186 cows. This tabulation shows a range of 48 days in the herd average calving interval with a mean for all cows of 401 days.

TABLE 1

Herd average calving intervals and yields for the first nine calving intervals

HERD NUMBER	NUMBER OF COWS	AVERAGE CALVING INTERVAL	AVERAGE 4 PER CENT MILK PER DAY	AVERAGE WEIGHT
		days	*pounds*	*pounds*
XIII	9	370	19.0	1,121
VI	4	375	14.4	981
IV	7	382	19.6	1,118
VII	2	382	13.9	
V	8	384	20.3	
XI	10	384	16.7	1,044
X	5	387	16.8	1,071
IX	11	391	16.5	1,079
II	34	403	16.7	1,017
VIII	12	404	18.5	1,185
III	22	410	17.8	973
I	43	411	16.7	1,046
XII	19	418	19.6	1,100
Average		401	17.5	1,061

The yield of 4 per cent milk shows considerable variation, but bears no particular relation to the length of calving interval. The average of all cows is 17.5 pounds of 4 per cent milk per day over the first nine calving intervals, which cover on the average

TABLE 2

Relation of length of calving interval to average yield of milk per day

The left-hand column shows the mid-points of the calving-interval groups by 20-day classes. The columns to the right show for the first 9 calving intervals of the 186 Red Danish cows, items as follows: n, the number of cows; Y_1, the average yield over the first calving interval in pounds of 4 per cent milk per day; Y_2, the same over the second calving interval; I_2, the mean length in days of the second calving interval; and so on to Y_9 and I_9. For example, dealing with the fourth calving interval there were 41 cows (n) with a calving interval of 350 days (column 1, line 5) that is, 340 to 359 days; the average yield of the 41 cows was 18.0 pounds of 4 per cent milk per day during the current calving interval (Y_4); and 18.2 pounds during the following calving interval (Y_5); the mean length of which was 402 days (I_5).

CLASSES	FIRST CALVING INTERVAL				SECOND CALVING INTERVAL				THIRD CALVING INTERVAL				FOURTH CALVING INTERVAL				FIFTH CALVING INTERVAL				SIXTH CALVING INTERVAL				SEVENTH CALVING INTERVAL				EIGHTH CALVING INTERVAL				NINTH CALVING INTERVAL	
	n	Y_1	Y_2	I_2	n	Y_2	Y_3	I_3	n	Y_3	Y_4	I_4	n	Y_4	Y_5	I_5	n	Y_5	Y_6	I_6	n	Y_6	Y_7	I_7	n	Y_7	Y_8	I_8	n	Y_8	Y_9	I_9	n	Y_9
270									1	21.9	16.5	385																						
290	1	11.9	14.0	355					1	19.1	19.5	425									1	23.5	17.5	385										
310	8	12.5	13.0	364	5	16.4	17.6	389	9	18.0	18.3	364	11	19.8	18.1	383	5	20.5	18.9	351	6	18.0	13.7	433	7	20.4	18.9	392	2	17.0	15.5	460	2	26.0
330	25	14.5	14.9	383	28	15.3	17.3	378	24	16.3	16.1	393	33	18.7	18.8	378	24	19.5	18.4	416	29	18.9	19.1	374	22	20.7	19.4	394	17	20.2	20.1	453	19	18.2
350	31	13.3	14.8	373	41	14.1	15.6	387	43	17.3	17.6	395	41	18.0	18.2	402	54	18.6	18.6	401	29	19.5	19.2	379	44	20.3	19.5	406	35	19.1	17.3	387	36	18.9
370	27	11.9	13.8	409	34	15.0	17.0	394	34	18.3	18.8	384	31	17.7	18.4	397	33	19.5	19.4	390	39	20.1	19.9	405	32	20.4	18.8	388	33	19.1	17.7	416	36	19.5
390	14	12.6	15.4	374	19	15.4	18.0	361	18	16.4	17.2	370	20	18.0	18.8	408	23	18.0	18.9	385	22	19.0	19.2	409	24	19.4	18.0	440	22	18.9	18.5	424	21	18.9
410	16	13.4	15.8	421	9	15.5	16.4	374	19	17.1	18.5	380	12	19.6	20.6	390	15	19.3	20.2	379	14	19.2	21.5	376	7	18.4	17.8	415	19	18.8	19.1	396	9	17.6
430	13	11.6	14.4	443	14	15.7	17.6	412	10	16.4	19.9	384	11	16.5	16.8	416	4	18.3	19.3	438	13	20.9	19.0	437	14	18.7	18.1	447	10	19.0	19.4	395	8	19.3
450	10	13.0	15.1	397	8	14.5	16.6	396	5	16.6	16.9	381	6	16.3	18.3	352	4	20.3	18.5	460	6	18.2	19.8	353	3	20.2	19.8	448	12	20.0	19.8	435	12	18.9
470	8	14.3	16.8	439	2	15.9	20.0	370	3	16.3	15.2	362	1	19.5	16.5	515	3	18.5	17.8	355	8	17.4	18.9	410	11	18.0	18.6	449	7	18.9	17.6	441	9	17.5
490	5	10.9	14.7	431	3	17.5	20.0	342	3	19.1	21.5	368	5	18.3	21.5	353	0				3	15.8	19.2	378	2	18.0	20.0	420	3	17.2	16.8	435	5	16.7
510	6	9.3	13.2	392	4	17.7	19.4	360	8	15.1	21.9	413	1	19.5	25.5	315	3	15.5	21.5	412	2	21.5	22.5	375	6	17.8	19.8	465	5	18.9	18.5	447	7	17.8
530	1	12.4	18.0	335	4	17.0	21.1	390	2	16.4	19.0	425	0				4	20.5	23.8	378	3	19.2	20.5	405	1	15.5	19.5	365	4	16.0	19.5	425	4	16.8
550	3	9.0	14.7	388	2	14.3	16.9	340	1	11.9	18.5	395	4	20.0	20.5	348	3	17.2	18.5	362	2	22.0	30.0	325	2	23.0	20.0	430	4	19.3	23.8	383	2	17.5
570	3	11.5	17.4	385	3	12.3	18.2	468	0				2	16.0	20.5	415	3	20.2	24.2	385	3	19.8	23.8	418	2	19.0	20.5	430	1	14.5	14.5	725	2	13.5

590	2	6.8	12.8	400	1	13 3	19 3	415	0				2	14 5	19 5	385	0				0				1	18 5	21 5	545	3	15 8	17 8	428	2	15.5
610	3	8 6	17.4	371	1	11 4	14 2	345	0				1	21 5	22 5	415	3	15 8	20 5	378	2	16 5	19 5	410	1	8 5	14 5	325	2	16 0	20 0	370	0	
630	3	11 6	15.7	398	4	13 5	15 2	583	1	18 2	19 5	385	2	18 5	22.0	545	0				1	20 5	16 5	465	0				0				3	19.8
650	2	10 4	18.7	485	2	13 7	17 8	365	1	15 9	19 5	555	0				0				2	15 5	17 0	590	1	13 5	15 5	425	0				2	15.5
670	1	9 0	12.0	375	2	15 5	19 8	360	1	19 5	23 5	385	1	16 5	20 5	475	0				0				1	14 5	23 5	365	1	16 5	22.5	365	1	20.5
690	2	10.2	18 7	405					0				0				2	17 0	22 0	355	0				2	14 0	15 5	365	0				2	20.5
710	0								1	16 5	20 5	405	0				0				0				0				4	16 3	20.8	495	1	6 5
730	1	15 2	18.5	545					0				0				1	11 5	19 5	425	0				0				0				2	16.5
750	0								0				1	16 5	22 5	355	1	17 5	27 5	385	0				1	13 5	16 5	665	0				1	22.5
770	0								0				1	12 5	28 5	375	0				0				1	15 5	20 5	385	0					
790	1	13 9	28.4	335					0								0				0				0				0					
810									1	9 4	18 5	625					0				0				0				0					
830																	1	8 5	17 5	425	0				1	18 5	19 5	395	0					
850																					0								1	21 5	17.5	325		
870																					0								0					
890																					1	16 5	21 5	345					0					
910																													0					
930																													1	19 5	24.5	385		

the first ten years of milking life. The average annual yield is therefore 6388 pounds of 4 per cent milk.

It is noticeable that the longer calving intervals occur in the larger herds, but the significance of this is not apparent. So far as can be seen from the herd averages of table 1 it should be permissible to throw the several herds together for the purpose of more detailed analysis.

The 186 records were sorted into classes on the first calving interval. For each of these classes were then computed: (n) the number of records in each class; (Y_1) the average yield per cow during the current calving interval, in pounds of 4 per cent milk per day; (Y_2) the average yield per cow during the following calving interval, likewise in pounds of 4 per cent milk per day; (I_2) the average length of the following calving interval in days. The records were then sorted on the second calving interval and a similar procedure followed; and so on to the ninth calving interval. The results are given in table 2.

TABLE 3

Calving interval, yield and fat percentage correlations

(For meaning of symbols see text, page 295)

n	COEFFICIENTS OF CORRELATION*							
	I_nY_n	I_nY_{n+1}	I_nI_{n+1}	Y_nY_{n+1}	Y_nI_{n+1}	I_nf_n	I_nf_{n+1}	f_nf_{n+1}
1	−0.286	+0.344	+0.124	+0.451	+0.031	+0.056	· 0.067	+0.733
2	−0.012	+0.121	+0.167	+0.558	+0.078	−0.068	−0.113	+0.774
3	−0.138	+0.211	+0.173	+0.583	+0.007	+0.089	+0.016	+0.811
4	−0.133	+0.195	+0.049	+0.618	−0.015	+0.020	+0.034	+0.794
5	−0.208	+0.230	−0.045	+0.485	+0.024	+0.029	+0.100	+0.761
6	−0.097	+0.033	+0.073	+0.482	−0.087	+0.130	+0.077	+0.746
7	−0.312	−0.049	+0.132	+0.464	+0.076	−0.137	−0.098	+0.791
8	−0.156	+0.166	+0.051	+0.336	+0.019	+0.123	+0.127	+0.846
9	−0.169					+0.034		

* The probable errors range from 0.014 to 0.049.

It is apparent from table 2 that as the calving interval increases the average yield per day tends to decrease during the current interval, and tends to increase during the following interval. But the relations are very irregular.

The various correlations have been worked out and are given in table 3. From this table we see that the correlation between calving interval and current yield per day is negative in each case; and, with one exception, it is positive when dealing with the following yield. In magnitude the coefficients are none very high and several are very low. There is a positive correlation between the length of one calving interval and that of the next, except in one case, but again the relation is not very pronounced. Such correlation as exists between the length of successive calving intervals might well be accounted for by the factor of herd practice rather than by any inherent tendency in the individual cow to a longer or shorter period in which conception may recur. The correlations, $Y_n I_{n+1}$, indicate that the yield during one lactation has no effect on the time of conception during the next lactation. Indirectly they may be taken to indicate also that rate of yield has no effect on the recurrence of conception at the time; and that there is no tendency to allow a longer calving interval to high yielding cows as compared with low yielding cows.

At this point we may interpose the results of correlating the average length of the first nine calving intervals with the average yield per day over the entire nine intervals. The coefficient, $r_{I_{1-9}Y_{1-9}}$, is 0.053 ±0.049 for the 186 cows and the regression is linear. Considered on this basis calving interval has no effect on yield; and, again, high yielding cows are not bred to freshen less frequently than low yielding cows.

Turning to the correlation between the yields of successive lactations it is seen that between the first and second intervals, $r = 0.451$; the coefficient increases regularly to the fourth and fifth intervals where $r = 0.618$; it then decreases regularly to the eighth and ninth intervals, where $r = 0.336$. We have here a "growth and senescence" curve with respect to stability of milk secretion. In this particular the prime of life is reached at an earlier age than occurs with respect to the yield of milk. In the present set of records the maximum average yield per day is found during the seventh calving interval.

On account of the great irregularities shown in table 2 it has seemed advisable to combine the several calving intervals to obtain a sort of average relation which may be more regular. In the relation expressed by $r_{l_n y_{n+1}}$ age influences may disturb the result as a measure of the influence of calving interval. For example, of two cows each dropping her first calf at two years, but one having a calving interval of one year, the other, of two years, it follows that the second lactation of one cow will begin at three years of age, the other, at four years of age. A similar condition will exist, of course, in subsequent calving intervals, but the effect on yield will be less marked. In dealing with $r_{l_n y_{n+1}}$ it was deemed expedient, therefore, to exclude the first calving interval.

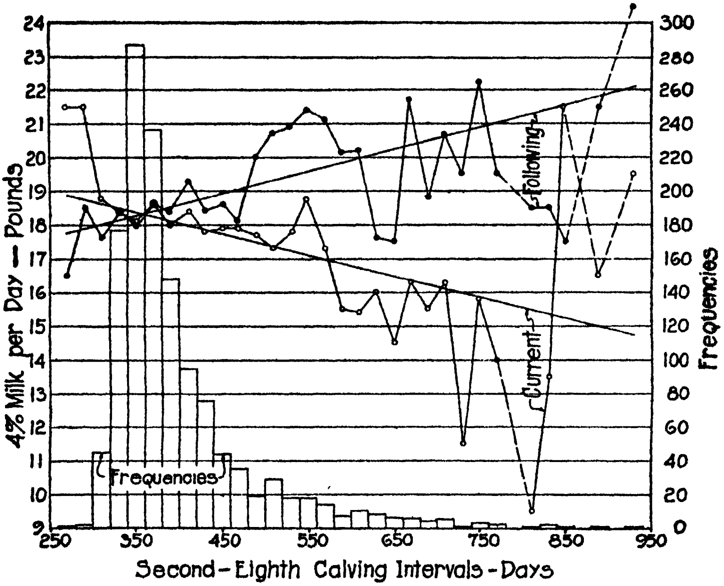


FIG. 1. SHOWING THE RELATION OF LENGTH OF CALVING INTERVAL TO AVERAGE YIELD OF 4 PER CENT MILK PER DAY OVER THE CURRENT CALVING INTERVAL AND OVER THE FOLLOWING CALVING INTERVAL, ALSO THE FREQUENCY DISTRIBUTION OF CALVING INTERVALS

Equations of fitted curves:

$$y = 20.61 - 0.00635\,x$$
$$y = 16.02 + 0.00652\,x$$

Where n takes successively values of two to eight inclusive, giving $186 \times 7 = 1302$ observations, the following correlations were found:

$r_{I_n Y_n} = -0.134 \pm 0.018$

$r_{I_n Y_{n+1}} = 0.142 \pm 0.018$

$r_{I_n I_{n+1}} = 0.088 \pm 0.019$

$r_{Y_n Y_{n+1}} = 0.527 \pm 0.014$

$r_{I_n Y_n \cdot I_{n-1}} = -0.148 \pm 0.018$

$r_{I_n Y_{n+1} \cdot I_{n+1}} = 0.156 \pm 0.018$

$r_{I_n Y_{n+1} \cdot Y_n} = 0.252 \pm 0.018$

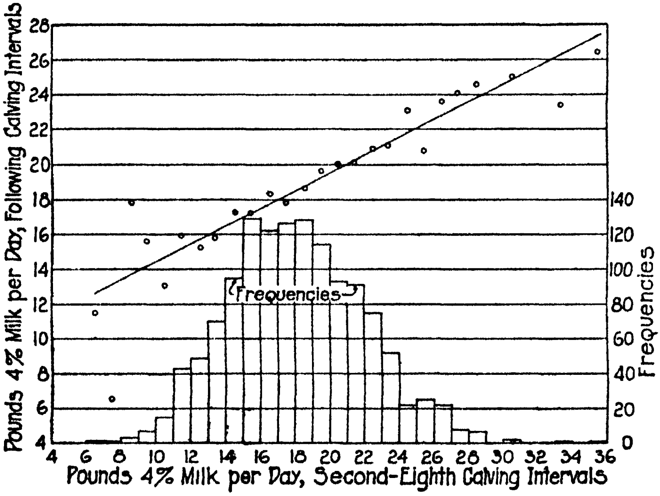


FIG. 2. SHOWING THE RELATION OF YIELD TO THE FOLLOWING YIELD, ALSO FREQUENCY DISTRIBUTION OF YIELDS, WHERE YIELD IS THE AVERAGE YIELD OF 4 PER CENT MILK OVER THE CALVING INTERVAL

Equation of fitted curve:

$$y = 9.39 + 0.510\,x$$

These correlations show that the length of calving interval is related to both the current yield and the following yield, and to about an equal degree. The observed regressions, treating the yield as dependent on calving interval, are shown in figure 1. The observations are still quite irregular, but the linear regressions derived from the constants may be regarded as describing the trends satisfactorily. The slopes are practically equal and in opposite directions. The data in this form fully support Matson's point in that what is gained currently by a short calving interval is "paid for" in the next lactation with a fine exactitude.

There is apparent in figure 1 a tendency for the observed curves to parallel each other. This is easily explained as due to the fact that the two observations for each calving interval class represent the same group of cows. If a group average is high this lactation it will be high also the next lactation. The correlation $r_{Y_n Y_{n+1}} = 0.527$ describes this characteristic, and simply indicates a certain degree of stability in productive ability. This feature is further shown graphically in figure 2.

DISCUSSION

The mean calving interval for the first nine lactations of these 186 cows is 401 days. Reference to figure one, however, shows the most common calving interval to be between 350 and 370 days. Evidently there is a pronounced tendency to have the cows freshen at yearly intervals. The very long calving intervals are probably due to some cause other than intent of management.

In case of the very long calving intervals there is undoubtedly a selection factor entering in with reference to yield. For example, a calving interval of 650 days means that the cow was not in calf a year after freshening. Unless such a cow were holding up exceptionally well in milk yield at this advanced stage of lactation she would be sold for beef. It seems to be clear, however, that if it is desirable to shift the season of calving, prolonging the calving interval up to 18 months need not adversely affect the average yield.

In connection with table 3 it may be permissible to mention a point somewhat apart from our subject. The point pertains to the suitability of the average yield over the calving interval as a measure of yield for biological study. The suitability of this measure as a matter of economics is evident. Is it equally suitable as a biological measure?

Bearing on this question table 4 is assembled from table 3 and from Gowen's (5) (6) work. Gowen's data are based on the records of Jersey cows in a single large herd and deal with the first eight months of lactation. His yield data represent actual milk production. The point of interest is as to the use of an absolute yield over a fixed portion of the lactation, and the use of an average figure over the entire lactation and dry periods. The higher correlations existing where a fixed portion of the lactation is used indicate that this is a more suitable measure of yield. The correlations for fat percentage show higher coefficients for the complete lactations of Langmack's Red Danish material than was found by Gowen for his eight months Jersey material.

TABLE 4

Coefficients of correlation between successive lactations bearing on the suitability of different measures of yield for biological analysis

LACTATIONS CORRELATED	LINE*	COEFFICIENTS OF CORRELATION	
		Yield	Fat percentage
First and second	a	0 451 ±0 015	0 733 ±0 009
	b	0 599 ±0 046	0 711 ±0 036
Second and third	a	0 558 ±0 013	0 774 ±0 007
	b	0 779 ±0 028	0 687 ±0 038
Third and fourth	a	0 583 ±0 012	0 811 ±0 006
	b	0 621 ±0 044	0 699 ±0 037
Fourth and fifth	a	0 618 ±0 012	0 794 ±0 007
	b	0 666 ±0 040	0 576 ±0 048

* The coefficients in line a are from the present paper. Those in line b are from Gowen, table 53 (5), and table 49 (6).

SUMMARY

Records published by Langmack (3) are considered with respect to the effect of length of calving interval on the average milk yield per day over the calving interval. The present data are confined to 186 Red Danish cows whose records in each case start with the cow's first calf and continue uninterruptedly through the nine

following calvings, all ten calvings being normal, that is, no abortion. Yield is considered on an energy basis in terms of 4 per cent milk.

There is uniformly a negative correlation between calving interval and yield over the current calving interval, and a positive correlation between calving interval and yield over the following calving interval. Throughout, the relation is irregular and the correlation coefficients are small in value. When the several calving intervals (excluding the first because the influence of age on yield is most marked at this stage of life) are thrown together the correlation between calving interval and yield over the current interval is $r = -0.134 \pm 0.018$; that over the following interval, $r = 0.142 \pm 0.018$. Thus, while there is a small gain from a short calving interval in the current lactation it is very exactly lost in the following lactation.

The calving interval distribution shows a mean of 401 days, but with a pronounced mode between 350 and 370 days. If it should be desirable to shift the time of year of calving it appears that the calving interval may be prolonged to 18 months without adversely affecting the average yield per day, considered over both the current and following intervals.

REFERENCES

(1) Gaines, W. L. 1927 Milk yield in relation to recurrence of conception. Jour. Dairy Sci., x, 117–125.

(2) Matson, J. 1929 The effect on lactation of the preceding calving interval and its relation to milking capacity, to age, and to other factors of influence. Jour. Agri. Sci., xix, 553-562.

(3) Langmack, P. V. F. P. 1921 Anden Beretning om Undersøgelse af de enkelte Koers Maelk. (Second report on the investigation of the milk of individual cows.) Beret. Forsøgslab. K. Vet. og Landbohøjskole (Denmark), 107.

(4) Gaines, W. L. 1928 The energy basis of measuring milk yield in dairy cows. Ill. Agr. Exp. Sta. Bul. 308.

(5) Gowen, John W. 1920 Studies in milk secretion. V. On the variations and correlations of milk secretion with age. Genetics, v, 111–188.

(6) Gowen, John W. 1920 Studies in milk secretion. VI. On the variations and correlations of butter-fat percentage with age in Jersey cattle. Genetics, v, 249–324.

DIETARY FACTORS INFLUENCING CALCIUM ASSIMILATION*

XIV. THE INFLUENCE OF MINERAL ACIDS AND SUGAR ON THE CALCIUM METABOLISM OF MILKING COWS

E. B. HART, H. STEENBOCK AND O. L. KLINE

From the Department of Agricultural Chemistry, University of Wisconsin, Madison

AND

G C. HUMPHREY

From the Department of Animal Husbandry, University of Wisconsin, Madison

It is becoming more and more evident that the metabolism of calcium in the lactating cow follows a rather definite path. First, in the early period of lactation, *especially with high milk flow*, the calcium assimilation from the digestive tract is insufficient to meet the needs of mammary secretion and the skeleton is drawn upon, with a negative calcium balance resulting. Second, with progressive lactation and a falling milk production a point is reached where calcium equilibrium is established; that is, sufficient calcium is absorbed from the digestive tract to maintain the calcium level of the blood without drawing upon the skeletal tissue. Third, on still lower production, and especially when the animal is dry, the assimilation of calcium from the digestive tract is sufficient not only to meet the needs of either a low milk production or calcium maintenance, but actually to supply sufficient calcium for active storage in the skeleton. In other words, in the period of low milk production or when dry the depleted calcium reserves of the bones are restored and the skeleton of the animal again put back, in respect to its mineral content, to the same place it started at the beginning of lactation. The above outline seems to portray the general situation, which may be modified, but not wholly corrected, by the level of calcium in the diet. Most

---

* Received for publication January 2, 1931. Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

of the published evidence indicates that in feeding legume hays or timothy hays fortified with lime salts, the losses of calcium to the animal will be reduced as compared with timothy hay alone, but even under these conditions calcium equilibrium or storage may not generally be established in the period of high milk production. The excellent records recently published by Huffman and associates (1) on calcium balances of lactating cows over entire lactation periods is experimental evidence for the above picture.

Just what forces are operative in the release of calcium from the bones during the early part of lactation is unsolved; but it is evident that there is some active compensatory mechanism, supplementing the calcium derived from the digestive tract, with the bones as the source of the calcium needed. Apparently these negative calcium balances in the early stages of lactation are without disturbance to the normal physiological function of the animal up to a certain point; probably a danger point is not reached because of the constantly lowering milk secretion with progressive lactation. For a number of years it has been our aim to stop, if possible, these calcium losses in the early part of lactation, and our later work on calcium metabolism of milking cows has been directed to that purpose.

In all previous work on this problem carried out at this station attempts have been made to influence favorably the assimilation of calcium in dairy cows during the early part of their lactation period through the use of such factors as vitamin D and calcium levels. In general it has been our experience that upon dry rations, with corn silage, the addition of calcium salts has not resulted in the production of positive calcium balances, although the balances may have been somewhat improved (2). This is in agreement with the work of Forbes and others (3). Ellenberger and Newlander (4) and Huffman and associates (1) report a better utilization of calcium salts added to the winter ration of dairy cows than observed by most of the other investigators in this field. Further, the increase in the supply of vitamin D, either in the form of cod liver oil, direct irradiation of the animal, or irradiated yeast, has also failed to convert negative calcium balances into positive calcium balances during the early period of lactation. On the other hand, there has been observed at this station, as well as by other investigators, that when the level of calcium is sufficiently high *fresh green plant tissue* favorably influences calcium assimilation. For example, in 1927 (5) in an experiment on calcium level and sunlight as effecting calcium equilibrium in milking cows it was found that these animals in the flush of milk production and yielding 50 pounds of milk per day could be kept in positive calcium balance when the ration consisted of about 40 pounds of green grass, 30 pounds of corn silage, and a grain mixture of 59 parts of yellow corn, 25 of wheat bran, 15 of oil meal, 1 of common salt, and a calcium salt addition. It was believed at that time, and subsequent to those experiments, that the factor favorably influencing the assimilation of calcium was the vitamin D of the green grasses. However, the fact that irradiated yeast (6) fails to influence favorably calcium assimilation in milking cows and that green grass does not contain much vitamin D excludes vitamin D as the dominating factor concerned in the green grass experiments, and makes it appear that there are other factors in the green plant tissue that favorably influenced calcium assimilation.

For a working plan we postulated the hypothesis that probably in the green grasses there were fairly high levels of fermentable sugars; that these sugars either by conversion into acids along the tract aided calcium assimilation by virtue of holding the calcium in solution, or that the sugars themselves increased the solubility of the calcium salts. It is well known that sugars may have the latter influence. We tested this theory by adding to the ration specific amounts of mineral acids such as hydrochloric acid or definite quantities of Cerelose, a glucose preparation made by the Corn Products Refining Company.

EXPERIMENTAL

Experiment 1

For our first experiment three Holstein cows in the early part of lactation were selected. They were fresh but a few weeks and gave between 40 and 45 pounds of milk per day. A fore-period of four weeks was run with a complete calcium balance on the animals. The ration consisted of 10 pounds of chopped alfalfa hay, 25 pounds of corn silage, and a grain mixture made up of 59 parts of yellow corn, 25 of wheat bran, 15 of oil meal, and 1 of salt. The grain was fed at the rate of 1 pound for about 3 pounds of milk produced.

In the second period of four weeks the same ration was continued, but to the alfalfa hay there were added daily either 115 or 230 cc. of 40 per cent hydrochloric acid—which would be equivalent to the addition of 46 or 92 grams of hydrochloric acid respectively. The addition of the acid was carried out as follows: The chopped alfalfa hay was placed in a wooden tub; the acid was diluted with water; and then the solution poured over the hay and stirred in. This mixture was prepared once daily for both the morning and evening feeding, allowing it to stand over night. The amount of water used to dilute the hydrochloric acid depended upon the amount necessary to moisten the hay.

This addition of the acid to the hay in no way disturbed the appetites of the animals. They consumed the ration with avidity and completeness. Two of the cows, nos. 2 and 3, in the second period of the experiment received the acid. The third cow, no. 1, received the basal ration fed in the fore-period, but with the substitution in the second period of 3 pounds of cerelose for 3 pounds of her grain ration; because of the reduction in the protein due to this displacement 0.6 pound of crude casein was added to her daily ration to compensate for the reduced protein.

The actual experiment began on October 24. Cow 1 was fresh October 4; cow 2, October 1; and cow 3, October 2. The udder of cow 3 became infected and it was necessary to displace her by another animal, which received the basal ration for but two weeks instead of four weeks, and the ration containing the acid for three weeks instead of four weeks. None of the cows were bred during the time of the experiment. Consumption of the ration was complete throughout the entire period.

TABLE 1

Cow 1—Ca balance and milk production—influence of sugar on calcium assimilation

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
Period I—basal ration							
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	573 85	225 45	14 37	813 67	819 96	+6 29	302 8
2	595 19	224 11	18 27	837 57	819 96	−17 61	301 0
3	644 33	205 55	14 18	864 06	819 96	−44 10	292 1
4	627 75	206 76	17 71	852 22	819 96	−32 26	279 4
Total	2,441 12		64 53			−87 68	
Period II—basal ration + sugar (3 pounds daily)							
5	565 49	203 72	9 73	778 94	806 63	+27 69	284 0
6	612 06	202 81	15 57	830 44	806 63	−23 81	283 6
7	558 09	196 81	13 34	768 24	806 63	+38 39	279 8
8	556 25	203 72	20 54	780 51	806 63	+26 12	284 0
Total	2,291 89		59 18			+68 39	

TABLE 2

Cow 2—Ca balance and milk production—influence of acid on calcium assimilation

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
Period I—basal ration							
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	527 87	222 25	8 24	758 36	825 21	+66 85	313 8
2	516 85	229 98	4 49	751 32	825 21	+73 89	318 6
3	604 78	221 23	6 78	832 79	825 21	−7 58	318 5
4	653 18	228 77	10 34	892 29	825 21	−67 08	325 1
Total. .	2,302 68					+66 08	
Period II—basal ration + 115 cc. strong HCl (daily for two weeks)							
5	562 18	228 27	10 23	800 68	825 21	+24 53	322 3
6	545 01	231 38	15 94	792 33	825 21	+32 88	328 8
Period III—basal ration + 230 cc. strong HCl (daily for two weeks)							
7	547.09	218 82	56 43	822 34	825 21	+2 87	319 2
8	546 48	221.60	74 37	842 45	825 21	−17 24	314 9
Total....	2,200 76					+43 04	

TABLE 3

Cow 3—Ca balance and milk production—influence of acid on calcium assimilation

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
Period I—basal ration							
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	593 28	221 40	9 91	824 59	825 21	+0 62	302 9
2	593 71	213 13	10 26	817.10	825.21	+8 11	293 4
Total ..	1,186 99					+8 73	
Period II—basal ration + 115 cc. strong HCl (daily for two weeks)							
3	632 40	211 87	8 62	859 89	825 21	−34 68	293 5
4	573 70	206 80	21 78	802 28	825-21	+22 93	288 3
5	582 66	199 71	29 33	811 70	825 21	+13 51	283 8
⅔ Total..	1,192 50					+1 76	

TABLE 4

Weekly titrations of milk, urine, and fece

WEEK	pH OF MILK—BASAL RATION			pH OF URINE—BASAL RATION			pH OF FECES—BASAL RATION		
	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3
1	6 95	6 80	6 90	8 25	9 80	8 80	7 12	7 13	7 38
2	6 80	6 95	6 72	7 85	9 80	9 10	8 40	8 77	7 15
3	6 75	6 85	6 70	9 25	9.80	7.80	8 25	8 45	7 02
4	6 95	6 85	6 72	9 60	9 80	8 15	7 95	8 05	6 67
	+ Sugar	+ Acid	+ Acid	+ Sugar	+ Acid	+ Acid	+ Sugar	+ Acid	+ Acid
5	6 83	6 92	6 75	9 05	8 35	7 75	7 20	7 30	7 03
6	6 80	6 87		8 90	8 18		6 85	7 90	
7	6 67	6 85		9 30	7 95		7 05	6 95	
8	6 95	6 87		9 15	7.50		6.80	6 53	

pH of urine—daily for weeks 7 and 8

cow 2—230 cc. acid daily
cow 3—115 cc. acid daily

COW	DECEMBER 6	DECEMBER 9	DECEMBER 10	DECEMBER 11	DECEMBER 12	DECEMBER 13	DECEMBER 14	DECEMBER 15	DECEMBER 16	DECEMBER 17	DECEMBER 18
2	6 75	7 95	6 42	7 57	8.32	6 67	7 14	6 95	7 50	7.32	7 53
3	7.65	8 15	7 95	7 75	7 76	7.97	7.85	7 67	7 75	7 87	7.75

In addition to the calcium balances secured, complete data were also secured at definite periods of the experiment upon the calcium and phosphorus content of the blood, upon the reaction of the milk, and upon the reaction of the urine and feces. By these determinations, especially the acid determination on the urine, information was secured as to the influence of the acid upon the general reaction of the excretory products. The pH was determined with the quinhydrone electrode. The data secured in this first experiment are brought together in tables 1, 2, 3, 4 and 5.

In the case of cow 1 there was a total negative calcium balance during the fore period of 87.68 grams of calcium oxide with 2441 grams of calcium oxide in the feces. In the period where she received the sugar a *positive* calcium balance of 68.39 grams prevailed and the calcium oxide in the feces amounted to 2291 grams or a reduction of about 150 grams. The data secured with this animal would indicate that there had been some favorable influence from the use of the sugar on the calcium assimilation.

TABLE 5

Blood Ca and phosphorus

Milligrams per 100 cc serum

	cow 1		cow 2		cow 3	
	Ca	Inorganic P	Ca	Inorganic P	Ca	Inorganic P
End of first period	10 44	6 66	9 58	5 03	10 91	5 03
Middle of second period	11 25	6 02	10 35	6 36		
End of second period	10 24	6 19	9 88	5 00	10 74	4 73

In the case of the two cows receiving the acid, no. 2 in the fore-period showed a positive calcium balance of 66 and 2302 grams of calcium oxide in the feces. In the acid ration period for the first two weeks she received but 115 cc. daily, while in the last two weeks she received 230 cc. daily. The effect of the acid was to increase the calcium excretion in the urine—which was to be expected. Her balance for the four weeks of acid feeding was positive by 43 grams, with 2200 grams of calcium oxide in the feces, or a reduction of about 100 grams over the fore-period. The fact that there was a marked increase in calcium excretion in the urine where the high amount of acid was fed, disturbed of course, a more favorable calcium balance of the animal as a whole. The calcium oxide in the urine rose from about 10 grams per week to 75 grams per week; and although there appeared to be a favorable influence of the acid on the assimilation of the calcium from the gut, this increased excretion in the urine gave a net result to the animal a little less favorable than where the acid was not added.

Practically the same situation occurred in the case of cow 3, although the periods for observation were shorter than in the case of cow 2. She showed a positive calcium balance during the first two weeks of the experiment on the basal ration. During the period where she received 115 cc. of acid per day the calcium in the urine increased. It rose from 8 grams per week to about 29 grams per week; but the net result of the addition of the acid was nevertheless a positive balance, although less positive than in the period where no acid was added.

These results from the addition of mineral acid would indicate some favorable influence upon calcium assimilation, but the necessity of using some of the base for the neutralization of the acid as it is excreted into the urine shows no favorable influence to the animal as a whole from such additions; whether an organic acid such as lactic or acetic would be more favorable in its effect through its partial or complete oxidation and consequent reduction of the losses of calcium through the urine is altogether possible.

The pH of the milk was in no way influenced by the acid additions. This is in conformity with past experiments at this station (7), where sulphuric acid has been added to the rations of dairy cows without influencing whatever the reaction of the milk.

The reaction of the urine was distinctly influenced by the acid additions. During the pre-acid period in the case of cow 2 the urine had a pH of 9.8. This was reduced to 8.2 during the period of low acid feeding and to 7.5 during the period of high acid feeding. In fact, during the last two weeks the daily titration of the urine varied in its pH, sometimes reaching as low as 6.4, but the average for the period was still on the alkaline side.

In the case of cow 1, where the sugar was added, there was no change in the pH of the urine. It remained on the alkaline side with a pH of 8 to 9 during the entire period.

The reaction of the feces was also apparently influenced by the acid treatments. During the fore-period the pH of the feces varied from 7 to 8; but during the acid feeding, and especially during the high acid treatment of the last two weeks, the pH of the feces decreased to 6.5. There seemed to be a somewhat similar tendency under the conditions of sugar feeding, but it was only slight.

The calcium and phosphorus content of the blood did not vary in the two periods.

Experiment 2

Because of the encouraging results from the use of sugar (cerelose) as a factor influencing calcium assimilation secured in experiment 1, in the spring of 1930 we set up another experiment which was limited wholly to testing further the influence of sugar on calcium assimilation in fresh milking cows.

Two Holsteins and one Ayrshire were chosen for this work. The experiment began on April 3. The cows had freshened as follows: No. 1, a Holstein, March 22; no. 2, a Holstein, February 17; no. 3, an Ayrshire, February 5. They were cows of four to six years of age and very quiet in the metabolism stalls. Their production was not high. The Holsteins produced from 35 to 40 pounds of milk per day, while the Ayrshire was producing from 45 to 50 pounds. Because of the somewhat lowered milk production and the necessity of giving the sugar theory a more crucial test the two Holsteins were placed upon a ration of timothy hay, corn silage, and grains. They were fed 10 pounds of timothy hay, 25 pounds of corn silage, and a grain mixture consisting of 64 parts of yellow corn, 10 of oil meal, 25 of wheat bran, and 1 of common salt. The Ayrshire was placed upon an

TABLE 6

Cow 4—Ca balance and milk production

(Timothy hay)

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
Period I—basal ration							
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	205 14	183 30	6.05	394 49	247 82	−146.72	244 7
2	185 26	185 70	9 27	380 23	247 82	−132.41	239 2
3	210 33	172 72	6 52	389 57	247 82	−141 75	236 3
4	230 11	161 59	6 92	398 62	247 82	−150 80	226 7
Total.	830						
Period II—basal ration + 3 pounds sugar daily							
5	239 22	162 85	9 51	411 58	257 16	−154 42	225.6
6	220 70	164 07	6.23	391.00	257.16	−133.84	217 7
7	216 11	156 07	4 89	377 07	257 16	−119.91	216 2
8	222 71	154 29	4 46	381 46	257 16	−124 30	212 4
Total	898						

TABLE 7

Cow 5—Ca balance and milk production

(Timothy hay)

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
Period I—basal ration							
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	171 82	230 28	7 30	409.40	247 82	−161.58	294 9
2	174 16	198 70	6 96	379.82	247 82	−132 00	277 0
3	228 97	177 63	6 85	413 45	247 82	−165 63	257 4
Total ..	574 95						
Period II—basal ration + 3 pounds sugar daily							
4	243 85	175.67	6 08	425.60	257.16	−168 44	252 9
5	253 21	171.57	4 79	429 57	257 16	−172.41	247.0
6	213 32	162.02	11.68	387.02	257.16	−129.86	227.3
7	235.59	151 75	14.24	401.58	257.16	−144.42	219.9
Total....	702 00						

alfalfa, corn silage, grain ration with the protein content of the grain mixture much less in order to compensate for the higher protein of the alfalfa hay. Her grain mixture consisted of 69 of yellow corn, 5 of oil meal, 25 of wheat bran, and 1 of common salt.

It was planned to run the basal rations for a preliminary period of four weeks. In the second four weeks there was a displacement of 3 pounds of grain for 3 pounds of sugar (cerelose) plus 0.6 pound of crude casein. Records were kept of calcium balance, calcium and phosphorus content of the blood, reaction of the urine, milk, and feces. The cows were quiet and contented throughout the entire experiment. They were not bred, and their consumption of the rations was complete during the entire eight weeks. The amount of grain each one was fed depended upon the milk produced and was approximately 1 pound of grain for 3 pounds of milk. The records of the calcium balances are displayed in tables 6, 7, and 8.

TABLE 8

Cow 6—Ca balance and milk production

(Alfalfa hay)

WEEK	CaO IN FECES	CaO IN MILK	CaO IN URINE	TOTAL CaO EXCRETED	TOTAL CaO INTAKE	Ca BALANCE	MILK PER WEEK
			Period I—basal ration				
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*pounds*
1	657 72	227 53	17 63	902 88	1,069 62	+166 44	331 9
2	671 19	248 39	25 13	944 71	1,069 62	+124 91	333 6
3	667 31	228 37	19 60	915 28	1,069 62	+154 34	320 4
4	695 08	215 19	12 74	923 01	1,069 62	+146 61	309 8
Total	2,691						
			Period II—basal ration + 3 pounds sugar daily				
5	729 19	224 81	7 88	961 88	1,081 82	+119 94	315 4
6	797 77	225 72	7 65	1,031 14	1,081 82	+50 86	312 7
7	805 26	232 22	5 57	1,043 05	1,081 82	+38 77	310 0
8	735 13	236 78	6 68	978 59	1,081 82	+103 21	305 0
Total	3,067						

With cow 1 (timothy hay) a negative calcium balance prevailed throughout the pre-sugar and the sugar period. The degree of loss of calcium was not reduced by the ingestion of 3 pounds of sugar daily. Eight hundred and thirty grams of calcium oxide were excreted in the feces during the pre-sugar period and 898 grams during the sugar period. In the case of cow 2 (timothy hay) results similar to those secured with cow 1 prevailed. In the three weeks of feeding the ration without sugar 574 grams of calcium oxide appeared in the feces, while in the first three weeks of the sugar period 709 grams of calcium oxide were excreted in the feces. In the case of cow 3 (alfalfa hay), where the ingestion of calcium oxide was approximately four times that of the timothy hay ration, positive balances appeared throughout the duration of the experiment. However, during the sugar feeding the amount of lime excreted into the gut was larger than the amount excreted during the period where no sugar was fed.

These experiments indicate that there was no positive influence on calcium assimilation resulting from mixing sugar (cerelose) with the ration; merely mixing sugar with the ration may not imitate the conditions that exist in a green plant cell, and until such imitations can be made one cannot positively know whether there is any value in the idea of the possible influence of sugar (cerelose) upon the assimilation of calcium. Recently Hunt and co-workers (8) have proposed the idea that the superior assimilation of calcium from green plant tissue as compared with dried plant tissue rests upon a more perfect dispersion of calcium in the green cell than in the dry cell, and that in this more highly dispersed condition the calcium is better assimilated. In opposition to that view, however, the fact must be recorded that Forbes (3) observed no beneficial effect on calcium assimilation when he added such soluble salts as calcium lactate or calcium chloride to a dry winter ration. It would seem that calcium salts as chlorides or lactates should be as readily absorbed as calcium salts in a dispersed state in the green plant cell.

In table 9 the record of the reaction of the milk, urine, and feces are given. It will be seen from these records that the ingestion of sugar had no effect whatever upon the reaction of milk. Further the reaction of the urine remained alkaline in the pre-sugar period as well as in the sugar period, and there was little if any influence from the sugar upon this reaction. The record of the reaction of the feces also gives no reason for believing that the sugar had influenced the reaction of the bowel excretions.

TABLE 9

Weekly titrations of milk, urine, and feces

WEEK	pH OF MILK—BASAL RATION			pH OF URINE—BASAL RATION			pH OF FECES—BASAL RATION		
	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3	Cow 1	Cow 2	Cow 3
1	6 80	6 65	6 81	8 15	8 52	8 20	5 88	6 01	7 05
2	6 84	6 75	6 75	8 68	8 20	8 34	6 15	5 95	7 10
3	6 90	6 67	6 85	8 00	8 15	8 55	5 75	6 15	6 15
4	6 87	6 65	6 75	8 50	8 35	8 03	6 05	5 95	6 85
	+ Sugar	+ Acid	+ Acid	+ Sugar	+ Acid	+ Acid	+ Sugar	+ Acid	+ Acid
5	6 80	6 75	6 82	8 65	7 85	8 12	5 67	5 98	6 82
6	6 82	6 69	6 97	7 30	8 03	8 00	6 25	6 23	6 75
7	6 75	6 75	6 83	7 51	7 10	8 37	6 20	6 20	6 62
8	6 84		6 90	8 35		8 12	5 85		6 65

TABLE 10

Blood Ca and phosphorus

Milligrams per 100 cc. serum

	COW 1		COW 2		COW 3	
	Ca	Inorganic P	Ca	Inorganic P	Ca	Inorganic P
End of pre-sugar period	11 76	7 39	10 78	7 05	10 88	6 65
End of sugar period. .	10 00	5 39	10 83	5 35	10 02	5 00

In table 10 is recorded the total calcium and inorganic phosphorus of the blood serum. It will be noticed that these results are very uniform in both periods and give no indication that there had been an influence on the calcium and inorganic phosphorus levels of the blood through the ingestion of sugar (cerelose).

While our data as a whole show no positive or consistent influence of sugar (cerelose) on the calcium assimilation in dairy cows, it should be pointed out that Robinson, Huffman, and Mason (9) did observe a favorable influence on calcium absorption in calves through the use of lactose. Also, in rats Bergeim (10) records a better utilization of calcium when the diet contained lactose than when the sugar was glucose, sucrose, maltose, or starch.

SUMMARY

In this paper data are presented on the influence of cerelose (glucose) and mineral acid (HCl) on the absorption of calcium by the milking cow.

1. There was no consistent and favorable influence on calcium assimilation in dairy cows through the daily addition of 3 pounds of cerelose (glucose) to a standard ration of hay, silage, cereal grains, and cereal grain concentrates.
2. The daily ingestion of 115 or 230 cc. of 40 per cent HCl increased the calcium excretion in the urine with a greater net loss of calcium to the animal than when no acid was fed. The calcium absorption from the intestine was slightly improved through the use of mineral acid.
3. What factor in green plant tissue operates in securing for the cow a better utilization of the calcium in the green plant tissue or added calcium salts is not solved by the technique used in the above experiments.

REFERENCES

(1) Huffman, C. F., Robinson, C. S., and Winter, O. B.: The calcium and phosphorus metabolism of heavily milking cows. Jour. Dairy Sci., 1930, xiii, no. 6, 432.

(2) Hart, E. B., Steenbock, H., Hoppert, C. A., Bethke, R. M., and Humphrey, G. C.: Dietary factors influencing calcium assimilation. III. The comparative efficiency of timothy hay, alfalfa hay, and timothy hay plus calcium phosphate (steamed bone meal) in maintaining calcium and phosphorus equilibrium in milking cows. Jour. Biol. Chem., 1922, liv, 75.

(3) Forbes, E. B., and associates: Mineral metabolism of the milch cow. Ohio Agricultural Experiment Station Bulletin 295, 1916; Mineral metabolism of the milch cow. Ohio Agricultural Experiment Station Bulletin 308, 1917; Mineral metabolism of the milch cow. Ohio Agricultural Experiment Station Bulletin 330, 1918; Mineral metabolism of the milch cow. Jour. Biol. Chem., 1922, lii, 281.

(4) Ellenberger, H. B., and Newlander, J. A.: Calcium and phosphorus balances of milking cows under varying conditions. Proc Soc. Animal Prod., 1928

(5) Hart, E B., Steenbock, H., Scott, H. T., and Humphrey, G. C.· Dietary factors influencing calcium assimilating. VIII. The calcium level and sunlight as affecting calcium equilibrium in milking cows. Jour. Biol. Chem , 1927, lxxi, 263

(6) Hart, E B., Steenbock, H , Kline, O L , and Humphrey, G. C.· Dietary factors influencing calcium assimilation. XIII. The influence of irradiated yeast on the calcium and phosphorus metabolism of milking cows. Jour. Biol. Chem , 1930, lxxxvi, 145.

(7) Sommer, H H , and Hart, E. B.: Grading milk by the acid test: Influence of acids in the ration on the acidity of milk. Jour. Dairy Sci., 1921, iv, no 1, 7.

(8) Hunt, C. H , Winter, A. R., and Miller, R C.: A possible factor influencing the assimilation of calcium Jour Biol. Chem , 1923, lv, 739

(9) Robinson, C S., Huffman, C F , and Mason, M. F : The results of the ingestion of certain calcium salts and of lactose. Jour. Biol. Chem., 1929, lxxxiv, 257.

(10) Bergeim, O. Intestinal chemistry VII The absorption of calcium and phosphorus in the small and large intestines Jour. Biol. Chem., 1926, lxx, 51.

CALCIUM ASSIMILATION AS INDICATED BY BONE ANALYSIS IN LONG-TIME EXPERIMENTS*

ARTHUR M. HARTMAN AND EDWARD B. MEIGS

Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

INTRODUCTION

The assimilation of calcium by dairy cows appears to be influenced by a number of factors. These are, the general character of the food, the quality of the roughage as influenced by the conditions of curing, the nutritive condition or individuality of the animal, the proportions of calcium and phosphorus in the diet, the quantity of calcium in the diet, and the milk yield.

Investigations of the question of calcium assimilation by dairy cows have been carried out almost entirely by means of balance experiments in which the intake and outgo of this element have been determined for varying periods of time and under various conditions of feeding. It seems desirable that the results of such experiments be supplemented by estimations of the assimilation of calcium by animals maintained under herd conditions and on a single dietary regimé for long periods of time.

It is the purpose of this paper to present the results obtained: (*a*) From calculations of the calcium assimilations of four cows fed continuously for periods of fourteen to thirty-two months on the same diet; (*b*) from the correlation of the figures for assimilation with the results of analyses and weights of bones obtained from one of these animals and from two other animals which had been on alfalfa hay for some time before death.

In reports of balance experiments carried out at this station, calcium assimilation for an experimental period has been defined (1) as the calcium contained in the food consumed minus the calcium contained in the urine and feces excreted during that period, due account being taken of intestinal lag. This value affords a very close approximation of the net intestinal absorp-

---

* Received for publication January 12, 1931.

tion. Percentage assimilation is this difference expressed as per cent of the intake.

EXPERIMENTAL

Long-continued feeding experiments at this station afforded the opportunity of studying calcium assimilation under practical conditions.

Four cows, three purebred Jerseys and one grade Guernsey, were fed rations consisting of timothy hay, a grain mixture and corn silage[1] for periods of fourteen to thirty-two months. Records were kept of the food consumed, the milk yielded, the weights of calves dropped, and the body weights of the animals.

The hay fed was certainly not better than average in quality and probably all of it would be classed as U. S. No. 2, some being a low-grade No. 2. The grain mixture, No. 55, was composed of corn meal 30, wheat bran 20, cottonseed meal 25, linseed meal 25, and salt 1 parts by weight.

For the whole period of timothy feeding, Cow 429 consumed 107.8 per cent of Savage requirements for total digestible nutrients; Cow 54, 112.6 per cent; Cow 456, 104.8 per cent; and Cow 450, 97.2 per cent. All four animals consumed considerably more digestible protein than required by the Savage standard.

No balances were determined on these four animals at any time during the experiment and the method of calculating the percentage calcium assimilation was as follows:

The calcium intake was determined from the percentages of calcium in the feeds and the quantities of the feeds consumed during the whole period; the outgo of calcium was calculated from the percentage of this element in the milk and from the total milk yield for the period and from the calcium contents and weights of the calves dropped during this time. The percentage assimilation was then calculated in accordance with the following equation:

$$\text{Percentage assimilation} = \frac{\text{Ca in milk} + \text{Ca in calves}}{\text{Ca in food}} \times 100$$

---

[1] Cow 450 received no silage.

TABLE 1

Composition of bones

BONE	WEIGHT OF BONE*			ASH			CALCIUM			PHOSPHORUS			ORGANIC MATTER			PROTEIN†			WATER		
	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17
	T.‡	A ‡	A.	T	A	A	T	A	A.	T	A	A.	T.	A.	A.	T	A.	A	T.	A.	A.
Dry basis																					
	grams	*grams*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Radius and ulna				53.2	52.2	58.6	20.8	20.2	22.7	9.51	9.37	10.44	46.8	47.8	41.4	25.0	23.3	24.9			
Tibia				50.0	47.3	55.8	19.5	18.5	21.5	8.97	8.50	9.94	50.0	52.7	44.2	22.9	19.9	23.4			
Metacarpus				56.9	56.1	61.3	22.2	21.6	23.7	10.07	10.06	10.90	43.1	43.9	38.7	25.9	24.9	25.4			
Metatarsus				58.4	56.6	62.8	22.9	21.8	24.3	10.43	10.11	11.18	41.6	43.4	37.2	26.5	25.3	26.2			
10th and 11th ribs				56.8	56.0	62.4	22.3	21.6	24.1	10.04	10.06	11.10	43.2	44.0	37.6	30.9	28.2	30.2			
Original moisture basis																					
Radius and ulna	722	902	640	46.2	46.3	46.8	18.1	18.0	18.2	8.26	8.31	8.34	40.7	42.4	33.1	21.7	20.7	19.9	13.1	11.3	20.1
Tibia	816	1,016‖	776	43.8	42.1	44.9	17.1	16.4	17.3	7.87	7.56	7.99	43.9	46.9	35.5	20.1	17.7	18.8	12.3	11.0	19.6
Metacarpus	260	340‖	259	50.2	49.6	50.0	19.6	19.1	19.4	8.88	8.89	8.89	38.0	38.8	31.6	22.8	22.0	20.7	11.8	11.6	18.4
Metatarsus	333	530‖	342	51.6	49.2	52.8	20.2	19.0	20.4	9.22	8.80	9.39	36.8	37.8	31.2	23.4	22.0	22.0	11.6	13.0	16.0
10th and 11th ribs	168§	203§	176§	46.2	47.1	51.4	18.2	18.2	19.8	8.16	8.45	9.14	35.0	37.0	30.9	25.2	23.7	24.9	18.7	16.0	17.7

* Average weight of the bones from both sides of the animal. Only one bone analyzed

† Nitrogen × 6.25.

‡ T. = timothy-fed; A = alfalfa-fed.

§ Average of weights of 10th, 11th, 12th and 13th ribs.

‖ Weight of bone from one side of animal only.

This method of determining the calcium assimilation presupposes no net change in the calcium stores of the body for the period of feeding. In order to get some idea of the extent to which the bones had been depleted of calcium after a long period of low-calcium (timothy hay) feeding, bones were obtained from one of these cows (No. 429)[2] which had been on timothy for thirty months. These bones were weighed and analyzed and the results were compared to weights and analyses of the same bones of two other animals which had been on alfalfa for some time before slaughter. The results of these analyses are given in table 1. Weights and analyses of separate bones are given and figures for phosphorus and protein are included. Cow 429, the timothy-fed animal, has been designated in the table by T; Cows 90 and 17, the alfalfa-fed animals, by A.

METHODS OF ANALYSIS

The bones were removed from the animal and cleaned superficially of attached tissue. They were then placed in a well-cooled refrigerator and remained there until taken out one at a time for thorough removal of tendons and remaining tissue. Much work was required to render the bones practically free of tissue and the small amount finally remaining could have had no noticeable effect upon the results of the analyses. After being cleaned they were returned immediately to the refrigerator and were kept there until weighed. The bones were always weighed after being in the refrigerator for some time and before weighing were allowed to come to about room temperature. Immediately after weighing they were shredded in a bone shredder and two large samples were weighed into 6-inch evaporating dishes. In most cases the two samples constituted practically the whole amount of shredded bone, and in all cases over half of it. The samples were dried for two or three days on a steam-heated sand bath; they were then allowed to come to an air-dried condition, ground finely in a small mill, placed in paraffin-sealed glass containers, and stored in the refrigerator until analysis was made. The bone prepared in this manner remains in excellent condition for quite a long period of time. Measurements of length, marrow cavity, etc., were made on some of the bones and in doing so change in moisture content was carefully determined and was corrected for in computing results of analyses.

---

[2] The death of Cow 429 occurred June 12, 1927. She had calved April 17, 1927, and yielded liberal amounts of milk for about six weeks. On May 30, 1927, she began to go off feed and down in her milk, and, in the course of a day or two, her milk yield and feed consumption were reduced to almost nothing and remained so until her death. On autopsy, she was found to have a rather general purulent inflammation, affecting the left lung, heart, diaphragm, liver, and mammary gland. (Quoted from an unpublished paper by E. B. Meigs on the performance of some animals on long time experiments.)

Ash. Ash was determined by heating approximately 10-gram samples in small porcelain evaporating dishes in an electric muffle furnace at 1200°F. until constant weight was obtained. The ash so obtained was white or very light gray in color. During the ashing process the samples were frequently removed from the furnace and stirred with a small glass rod.

Calcium. The evaporating dish containing the ash was immersed in a solution of hydrochloric acid in a large beaker, warmed to hasten solution, cooled and made to a large volume (2000 cc.). Calcium was determined on aliquots of this solution by the method outlined in previous publications from this laboratory (2) (3), except that hydrochloric acid was displaced completely by sulphuric in the solution to be precipitated. This modification has been in use for a number of years at this laboratory and has the advantage of removing the possibility of interference of chlorides in the titration of the oxalate with $KMnO_4$.

Phosphorus. Phosphorus was determined as magnesium pyrophosphate by the method outlined in the article referred to above (2). Approximately 5-gram samples of bone were ashed in the wet way and diluted with water to about half the volume to which they were to be made. The suspended calcium sulphate was filtered off and washed, and the combined filtrate and washings were brought to 500 cc. in a volumetric flask. Aliquot portions of this solution were used for analysis.

Nitrogen. Nitrogen was determined on 2 to 4 grams of bone by the Kjeldahl-Gunning-Arnold official method (4).

Protein. Protein was calculated by multiplying the nitrogen by 6.25. This factor is sufficiently close for the purposes of this paper.

Organic matter. Organic matter was calculated by subtracting the per cent ash in the water-free bone from 100.

TABLE 2

Average bone analyses

	DRY BASIS						ORIGINAL MOISTURE BASIS					
	Long bones			Ribs			Long bones			Ribs		
	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17	Cow 429	Cow 90	Cow 17
	T*	A*	A.	T	A	A	T	A	A	T	A	A.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Ash	54.6	53.0	59.6	56.8	56.0	62.4	48.0	46.8	48.6	46.2	47.1	51.4
Calcium	21.4	20.5	23.1	22.3	21.6	24.1	18.8	18.1	18.8	18.2	18.2	19.8
Organic matter	45.4	47.0	40.4	43.2	44.0	37.6	39.8	41.5	32.8	35.0	37.0	30.9

* T. = timothy-fed; A = alfalfa-fed.

TABLE 3

Histories of cows

COW NUMBER	AGE AT DEATH	TIME ON RATIONS	TOTAL MILK YIELD	DRY FOR:
	years	*months*	*kgm*	*months*
429*	9	30	8,260	6½
90†	5¾	34	5,613	4
17‡	16	32½	4,137	8

* At death had completed first one and one-half months of a lactation.

† Slaughtered at end of nine months of a lactation; bred one month before slaughter.

‡ Dry for four to five months before slaughter; not pregnant.

Water. Water was determined by heating at 70°C. in vacuo (20 to 30 mm.) to constant weight.

In table 2 are given average results of analyses of long bones and of ribs and in table 3 some facts in the histories of these animals. Although moisture changes in handling the bones were determined and controlled as carefully as possible throughout, the values on the dry basis are to be regarded, perhaps, as the more accurate of the two sets of figures in tables 1 and 2.

In the last thirty months of her life Cow 429, a purebred Jersey, was on timothy hay for this entire period. Of the last thirty-four months of her life Cow 90, a grade Guernsey, was on alfalfa for the first ten, timothy for the next fifteen, and on alfalfa for the last nine. Cow 17, a grade Jersey, was, during the last thirty-two and one-half months of life, on alfalfa for the first twenty-eight and one-half, on timothy for the next one and one-half, and on alfalfa for the last two and one-half. Cow 429 at death had completed the first one and one-half months of a lactation, No. 90 at slaughter had completed nine months of a lactation, and No. 17 was dry for from four to five months before slaughter.

TABLE 4

Bone weights as percentages of body weights

	COW 429	COW 90	COW 17
	per cent	*per cent*	*per cent*
Long bones .	0 491	0 576	0 545
Ribs . .	0 155	0 168	0 191

From table 3 it is seen that No. 429 gave much more milk during this time than either of the alfalfa cows.

The figures (table 2) for bone analyses show throughout that there is little difference in the content of ash, calcium, and organic matter in the bones of No. 429, the timothy-fed cow, and No. 90, the alfalfa-fed cow, the former having, if anything, slightly higher values for ash and calium. On the dry basis, No. 17, the other alfalfa-fed cow, is distinctly higher in ash and calcium and lower in organic matter than are Nos. 429 and 90. On the original moisture basis, in the case of the long bones No. 17 exhibits about the same content of ash and calcium, this being accouted for by the higher water content of the long bones of this animal. The figures for the ribs of this cow are higher due to the water content being about the same as the other two cows. Cow 17 was, however, quite an old animal and it is well known that the mineral content of the bones increases somewhat in old age.

The results show that the long period of low-calcium feeding on timothy hay has brought about little if any change in the relative proportions of ash, calcium, phosphorus, and organic matter in the bones.

It may be supposed that the ratio of bone weight to body weight would give some indication of the loss of calcium from the skeleton, on the theory that the entire bone material disappears upon depletion of the minerals. The ratios of bone weight to body weight have, therefore, been calculated and are given as percentages in table 4. The figures for the long bones were obtained by dividing the sum of the average weights of the two metacarpi, metatarsi, radii and ulnae, and tibiae by the body weights and those for the ribs were obtained in a similar manner from the weights of the 10th, 11th, 12th, and 13thribs. The figure for the body weight of Cow 429 used in this calculation was obtained by averaging the monthly weights of the entire period on timothy hay, eliminating the four months before and the two months after calving. The body weight of Cow 90 was obtained in a similar manner from the monthly weights for two years previous to slaughter. That of Cow 17 was calculated from the first twenty-four of the thirty months preceding slaughter; these earlier monthly weights were used because it was noted that the body weights of the six or seven months immediately preceding death were low. If these low weights had been used in the calculations, the values of the ratios given in table 4 would have been increased.

The values of the ratio of bone weight to body weight in the case of Cow 429, the timothy-fed animal, are seen to be 10 to 20 per cent lower than those of the two alfalfa-fed cows. Considered on the basis of the theory upon which these figures have been calculated, these values suggest that the skeleton of No. 429 was depleted of calcium and of other inorganic elements to the extent of 10 to 20 per cent as a result of her long period on the timothy hay ration.

In table 5 are given the percentages of calcium assimilation of the four cows which were on the timothy rations for long periods. The last two columns show the number of lactations that fell within

TABLE 5

Per cent calcium assimilation of cows on timothy hay rations

$$\text{Per cent Ca assimilation equals } \frac{\text{Ca in milk plus Ca in calves minus Ca lost from bones}}{\text{Ca in food}} \times 100$$

COW	BREED	TIME ON RATIONS	DRY PERIOD	AVERAGE DAILY FEED CONSUMED			CALCIUM INTAKE FOR PERIOD				CALCIUM OUTGO FOR PERIOD			ASSIMILATION WHEN LOSS OF BODY Ca IS:			MILK YIELD		PERIOD OF LACTATION
				Hay	Grain	Silage	In hay	In grain	In silage	Total	In milk	In calves	Total	None	15 per cent	25 per cent			
		mos.	*mos*	*kgm*	*kgm.*	*kgm*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm.*	*kgm*	*kgm.*					*kgm.*	*months*
429	P. B. Jersey	30	6½	3 60	6 39	6 38	9 575	9 270	5 718	24 563	10 821	0 61	11 431	46 5	42 5	39 8	(1)	2,666	Last 9 of 12½
																	(2)	4,826	Entire 13
																	(3)	768	First 1½
																	Total	8,260	
54	Grade Guernsey	27	5	3 47	6 07	6 49	8 162	7 937	5 235	21 334	5 852	0 36	6 212	29 1	22 8	18 6	(1)	1,702	Last 9 of 13
																	(2)	4,149	Entire 13
																	Total	5,851	
456	P. B. Jersey	32	5½	3 33	4 90	5 11	9 204	7 575	4 884	21.663	6 454	0 61	7 064	32 6	28 0	25 0	(1)	1,090	Last 9 of 12½
																	(2)	2,309	Entire 13
																	(3)	1.211	First 4½
																	Total	4,610	
450	P. B. Jersey	14	3½	3 93	4 71	0	4 760	3 190	0	7 950	2 423	0 21	2 633	33 1	26 1*	21 4†	(1)	1,731	Entire 10½

* Seven and one-half per cent loss of body Ca.
† Twelve and one-half per cent loss of body Ca.
Since time of No. 450 on ration was only approximately one-half that of other cows, this assumes a linear relation between loss of body calcium and time.

the feeding period, the portion of the lactation, the milk yield for that portion, and the total milk yield for the whole period. Again it is to be noted that Cow 429 secreted much more milk than the other cows on the rations for similar periods of time.

The per cent calcium assimilation was calculated in accordance with the equation at the top of the table. The calcium content of the hay fed was determined. The calcium content of the grain No. 55 fed was not determined. The figure used, 0.159 per cent Ca, is the average of maximum and minimum values of the analyses of two separate lots of Grain 55 fed during a balance experiment carried out at this station. The maximum figure was 0.162 and the minimum 0.156 (1, p. 846). The percentage of calcium in the corn silage fed was not determined. The figure used, 0.0982 per cent Ca, is the average of the maximum and minimum values of analyses of corn silage fed during a balance experiment carried out in this laboratory. The maximum value was 0.1037 and the minimum 0.0926 (3, p. 495).

Henry and Morrison (5) give the calcium content of timothy hay as 2.5 parts CaO per thousand. This would be equivalent to 0.18 per cent Ca, while the average Ca content of the hay fed to our cows ranged from 0.284 to 0.291 per cent for the different cows. The figures given by Henry and Morrison for the calcium content of the constituents of our grain No. 55, would make the calcium content of this mixture 0.172 per cent, which is somewhat higher than our figure, though not nearly enough to compensate for the difference in the timothy figures. No figures are given by Henry and Morrison for the calcium content of corn silage, but this constituent supplied only a rather small proportion of the total calcium content of our rations. From the considerations which have just been given it does not seem likely that the calcium content of our rations has been underestimated.

The calcium content of the milk of No. 429 was 0.131 per cent (February, 1925); that of No. 54, 0.100 per cent, this figure being an average over 29 periods during two balance experiments carried out on this animal (1). The calcium contents of the milks of Nos. 456 and 450 were not determined. The figure used was 0.140 per cent Ca. This is an average of the values for the calcium content of the milk of Cow 412, 0.142 per cent Ca, that of No. 441, 0.146 per cent Ca (averages of 7 and 6 periods, respectively, during a balance experiment on these animals (6)), and that of Cow 429, 0.131 per cent Ca. All five of these cows are purebred Jerseys of the Beltsville herd.

The calcium content of a calf at birth has been taken as 1.28 per cent. This figure is the average of the values (1.169, 1.261, 1.258, 1.437, 1.273) for five Jersey calves analyzed at the Missouri Agricultural Experiment Station (7). This average value has been used in computing the calcium content not only of the calves of the three Jersey cows, Nos. 429, 456, and 450, but also that of the calves of No. 54, a grade Guernsey.

The calcium content of the body of a mature cow is approximately 1.5 per cent. This figure was calculated by utilizing, with certain approximations, the values of the Ca content of steers at the ages of forty, forty-four, and forty-eight months, slaughtered and analyzed at the Missouri Agricultural Experiment Station (7). This value (1.5 per cent) is practically identical with that calculated from figures given by Hart (8). From data of the Rothamsted Experiment Station, Hart estimated that there were 24.2 pounds of CaO in the body of a Holstein cow weighing 1150 pounds.

In table 5 are given three values for the percentage calcium assimilation, computed by:

1. Assuming no loss of body calcium during the feeding period.
2. Assuming a loss of 15 per cent of body calcium. This is approximately the loss indicated by the decrease in the weight of the bones of Cow 429 as presented in table 4.
3. Assuming a loss of 25 per cent of body calcium. This is undoubtedly a liberal allowance and, other things being equal, the assimilations calculated on this basis are very probably to be regarded as minimum values.

The writers realize keenly the many sources of possible error in the results which have been given. But, in considering these results, and the possible errors to which they are subject, it must be remembered that one of the main objects of investigating calcium metabolism is to determine what proportion of her calcium intake a cow can utilize for the production of milk and calves on the average, when she is kept under ordinary practical conditions for a long period year in and year out. This question has been investigated in the past chiefly by means of balance experiments, and it must be pointed out that neither the nature of such experiments nor the results that have been obtained from them give any warrant for supposing that they furnish a flawless method for answering the question under consideration. In order to apply their results to answering this question, it must be assumed that results obtained in short periods during which the animals cannot be kept under normal conditions are representative; the justification for this assumption will depend largely on how far the results of different balance experiments agree with each other.

Unfortunately there is no satisfactory agreement among the results of balance experiments. Not only do groups of results obtained by different investigators disagree considerably with one another, but it often happens that different animals studied by the same investigators under apparently identical conditions of feeding and treatment show wide differences in their calcium assimilation (1). It seems justifiable, therefore, to regard the results of our experiments as a valuable check on the results of balance experiments.

The values for calcium assimilation in table 5, calculated on the basis of 15 per cent loss of body calcium, are somewhat higher and those calculated on the basis of no loss of body calcium are considerably higher than those which have been obtained in most[3] balance experiments (about 20 per cent) (1). The difference between individual animals is strikingly evident. Cow 429 assimilated almost 40 per cent of her food calcium, even allowing for a loss of 25 per cent of her calcium stores. It seems, therefore, that good cows may be capable of assimilating a relatively large proportion of their dietary calcium under these conditions.

The basis upon which the figures for 15 per cent loss of boyd calcium have been calculated was, as stated previously, the lower bone weight-body weight ratios for Cow 429. Obviously, this ratio is greatly influenced by the degree of fatness or fleshiness of the animal. No estimates of the relative amounts of fat or of flesh of these cows were obtained. But even if the ratios were higher for No. 429 than for the two alfalfa cows, or if the ratios were the same for all three and the theory of the disappearance of the entire bone material upon depletion of the minerals is untenable, this only further emphasizes the fact that the values for calcium assimilation are higher than those obtained in most balance experiments, since then the only determining factor would be the change in composition of the bone. This was found to be little if at all altered by long periods on timothy hay and hence the values of percentage calcium assimilation calculated by assuming no loss of body calcium would be the only basis for comparison with figures from balance experiments.

---

[3] Ellenberger of the Vermont Station has recently reported (9) percentages of assimilation of 50 per cent and over on rations containing timothy hay unsupplemented with minerals.

With regard to the content of mineral matter in bone, age obviously plays a rôle and it might be contended that the bones of Cow 429 (nine years of age) would, if she had been on a high calcium diet (alfalfa hay), have shown a larger mineral content than those of No. 90 (five and three-quarter years of age). However, Cow No. 17 (sixteen years old), a considerably older animal, had a mineral content greater than No. 90's by only about 12 per cent, calculated on the dry basis. It is scarcely to be expected that there would be anywhere nearly as large a difference between Cow No. 429 (aged nine years) and Cow No. 90 (aged five and three-quarter years). A similar conclusion may be reached with respect to the possible effect of the stages of lactation at which the deaths of the animals occurred, or of pregnancy, on the mineral content of the bones. The bones of Cow No. 17, the alfalfa-fed animal, were obtained after she had been dry for from four to five months. Their mineral content was only about 8% greater than those of Cow No. 429, the timothy-fed animal, whose bones were obtained at the end of one and one-half months of lactation. The only one of the three cows which could have been pregnant was Cow No. 90; she was bred just one month before slaughter.

That the determinations of the chemical elements contained in bone were accurate is to be inferred from the fact that the Ca:P ratios in the bones of all the animals used in this investigation were close to the normal ratio. From data given by Hammarsten (10) the ratio of Ca:P by weight in bone is about 2.15. According to the figures given in table 1 (dry basis), this ratio did not in any case rise as high as 2.23 or fall as low as 2.14 in the bones of the animals studied.

The results agree with those of previous investigations in showing that the Ca:P ratio in the bones of animals cannot be changed by feeding them a ration low in calcium. Curiously enough the proportion of calcium to phosphorus was higher on the average in the case of Cow No. 429, which had been on the low calcium ration, than in that of either of the other two cows. In the bones of Cow No. 429 the Ca:P ratio was about 2.20, while in those of Cow No. 90 and Cow No. 17, it was only 2.16 and 2.17, respectively.

SUMMARY

Four cows were maintained under herd conditions on a ration of timothy hay, a grain mixture, and corn silage for periods of fourteen to thirty-two months.

Analyses were carried out on the bones of one of these cows (thirty months on timothy) and on those of two other animals which had been on alfalfa hay for some time before slaughter. Calculations have been made of the percentages of calcium assimilated by the four cows on timothy hay.

1. The results indicate that the relative amounts of ash, calcium, phosphorus, nitrogen, and organic matter in cows' bones are little if at all altered by long periods on rations low in calcium, even when a considerable amount of milk is given in such periods.

2. The results suggest that the treatment above described reduces by from 10 to 20 per cent the total weight of the bones, and, therefore, the amounts of calcium and phosphorus in the body.

3. The results indicate that, even on a mediocre roughage, the calcium assimilation tends to be somewhat higher in long periods under natural conditions than it has been in most balance experi-

ments, and that, in the case of good cows, it may be much higher than it has been in most balance experiments.

REFERENCES

(1) Meigs, E. B., Turner, W. A., Harding, T. S., Hartman, A. M., and Grant, F. M.: Calcium and phosphorus metabolism in dairy cows. Jour. Agri. Res., xxxii, 833 (1926).

(2) Meigs, E. B., Blatherwick, N. R., and Cary, C. A.: Contributions to the physiology of phosphorus and calcium metabolism as related to milk secretion. Jour. Biol. Chem., xxxvii, 1 (1919).

(3) Meigs, E. B., Blatherwick, N. R., and Cary, C. A.: Further contributions to the physiology of phosphorus and calcium metabolism of dairy cows. Jour. Biol Chem., xl, 469 (1919).

(4) Official and Tentative Methods of Analysis of the A. O. A. C., Washington, D. C., 1920.

(5) Henry, W. A., and Morrison, F. B.: Feeds and Feeding, 18th Ed., 1923, p. 752. Madison, Wisconsin

(6) Turner, W. A., Harding, T. S., and Hartman, A. M.: The relative assimilation by dairy cows of clover and alfalfa hays and of rations of different calcium and phosphorus content. Jour. Agri. Res., xxxv, 625 (1927).

(7) Hogan, A. G., and Nierman, J L.: Studies in animal nutrition. VI. The distribution of the mineral elements in the animal body as influenced by age and condition. Missouri Agri. Expt. Station Research Bulletin 107 (1927).

(8) Hart, E. B., McCollum, E. V., and Humphrey, G. C : The rôle of the ash constituents of wheat bran in the metabolism of herbivora. Amer. Jour. Physiol., xxiv, 86 (1909)

(9) Ellenberger, H. B., and Newlander, J. A.: Calcium and phosphorus balances of milking cows under varying conditions. Proc. Amer. Soc. of Animal Production, p. 25, January, 1929.

(10) Hammarsten, O.: A Text-book of Physiological Chemistry. Ed. 7, p. 552. Translated from the 8th German Edition by J. A. Mandel, New York and London.

PROPERTIES OF SOME ACID CASEINS*

R. W. BELL AND S. P. GOULD

Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.

The claim has been made that imported casein is superior to the domestic product and is, therefore, preferred by paper-coaters, the principal users of casein. In order to obtain data on the properties of domestic casein, a number of samples were analyzed and tested within a few days after their receipt from the manufacturers. Data on the first ten of the samples received are presented here. In a majority of the samples the dry casein had evidently been placed in the mailing cases as it came from the trays. The samples were all ground to a uniform size so that practically all of the particles would pass through a 20-mesh sieve.

Appearance. The appearance of the samples after grinding to a uniform size was much the same except for a small variation in the amount of dirt and burned particles. The color was good. Particle size affects color. Differences in this property may best be determined by examining solutions of the samples.

There was no indication that any of the samples had deteriorated because of long storage.

Fat. The method described by Sutermeister (1) for the estimation of fat in casein was used in these tests. This method is a modification of the Babcock method and is much more rapid than procedures which include the use of organic solvents. The condition of the fat at the end of the tests indicated that the concentration and amount of sulphuric acid used was sufficient to dissolve the casein without charring the fat.

Moisture. Approximately 1.0 gram was weighed into a small flat platinum dish and dried in a vacuum oven for five hours at 98.0°C. The weight of the dry casein was obtained and the sample was then used for ash determinations.

Ash. If the sample is low in ash the addition of calcium in the

---

* Presented at the Annual Meeting of the American Dairy Science Association, Ames, Iowa, June, 1930. Received for publication January 13, 1931.

form of calcium acetate is necessary to prevent volatilization of organic phosphorus during ashing, as shown by Shaw (2). Therefore, in order to prevent any possible loss of phosphorus, 2.0 cc. of approximately M/10 calcium acetate solution (0.0116 gram CaO) was added to each sample before charring it over a micro-burner. Care was taken to prevent the casein from bursting into flame. One cubic centimeter of C. P. concentrated nitric acid was added to each sample. The samples were gently heated before placing them in the muffle. It is difficult to get a white ash without the action of the acid.

Strength. The method used for determining the strength of the casein was essentially the same as the method for strength determinations described by Sutermeister (3). Two or more grams of borax were used as the solvent. The solution was tested in each case with phenolphthalein to be sure that it was alkaline. The method is an empirical one. The numbers 6 to 12 denote the strength of the casein and refer to the grams of casein in each coating preparation. The greater the adhesiveness of the casein the smaller is the number denoting its strength.

A standard paper was used for all the tests. It was a rosin-sized, 100-pound machine-finish book paper obtained from the Government Printing Office.

Sufficient china clay was obtained to last through the determinations so that any variation due to a difference in the clay was eliminated.

Viscosity. A great deal of work has been done by a number of investigators on the viscosity of casein solutions. The methods employed have involved the principle of time of efflux from a standard orifice, or else the torsion of a wire caused by the resistance of the casein solution to the rotation of a plunger. Some observers claim to be able to judge the viscosity or fluidity of casein solutions by simple observation, but it is impossible to do this with any degree of certainty because an opaque creamy solution may look viscous and have a low viscosity expressed in absolute units, whereas, a transparent watery looking solution will often have a comparatively high viscosity.

The great variety of methods employed to determine the viscosity of casein solutions makes it impossible to compare the work of one experimenter with that of another. Sutermeister (2) states, "No one has yet made a study to find out which casein solutions, if any, are *viscous* and whether the more important solutions are to be regarded as *plastic* or perhaps of a still more complicated flow character requiring three or more parameters for its description."

There are many methods of determining the viscosity or consistency of a fluid or mushy material. One of these is the burette consistometer. The fundamental principle of its use is to make a series of observations at different rates of flow by permitting the hydrostatic head to vary. The burette consistometer requires no external source of pressure and serves as a convenient means for obtaining flow-pressure data on materials that are not too stiff. This instrument has been used by Cook (4) and Auerbach (5). Herschel and Bulkley (6) describe Auerbach's apparatus and also report a method of calibrating the burette consistometer and the degree of concordance attainable.

The apparatus and methods used in this work for making determinations of the consistency of the casein solutions were essentially those described by Herschel and Bulkley. The lower end of a 50.0 cc. burette was cut off just above the beginning of the constriction near the stop-cock and suspended in a water bath as shown in figure 2 of Herschel and Bulkley's article. It was then calibrated by means of a cathetometer at each 5.0 cc. mark. The capillary tube was inserted in the lower end of the burette and held in place by a rubber stopper in such a manner that the upper end was always even with the 40.0 cc. graduation on the burette. This prevented cooling or warming of the liquid in the capillary since most of the capillary tube was surrounded by the material being measured. It was decided to make readings at three different heads and to take the time required for the liquid to flow from the 0.0 to 5.0, 10.0 to 15.0, and 20.0 to 25.0 cc. graduations. The average heads were calculated by the formula

$$h = \frac{h_1 - h_2}{\log_e\left(\frac{h_1}{h_2}\right)}$$

where h_1 is the initial and h_2 is the final head. The average heads were 56.31, 45.41 and 34.49 cm. respectively. The length of the capillary was 15.6 cm. and the diameter 0.102 cm. It took from about one to two and one-half minutes for the 5.0 cc. portions to flow from the capillary.

The equation used by Bateman and Sharp (7) was employed for determining the consistency of the casein solutions. In this equation, ξ, the consistency of a plastic substance, corresponds to N, the viscosity of a liquid.

$$\xi = \frac{\pi r^4 g(P - p)t}{8\ lv}$$

where r is the radius of the capillary, l its length, v the volume flowing through the capillary in time t, P the total pressure in grams per square centimeter producing the flow, p the yield value pressure and g the gravitational constant. The only difference between the fundamental equation for viscous flow and that for plastic flow is the substitution of $(P\text{-}p)$ in the latter for P in the former. If p equals zero the substance is viscous; if p has a value greater than zero the substance under investigation is plastic.

It was decided, for reasons which will be described later, to use a 9.0 per cent casein solution with sodium hydroxide as the solvent. The average specific gravity of a large number of the solutions was found to be 1.02, the variation between samples being slight. The value of P for the greatest head, therefore, becomes 56.31 grams per square centimeter (the average head between the 0.0 and 5.0 cc. graduations) times 1.02.

Measurements were made at 30.0°C.

It was found that the value for p at the different heads was zero, so that the values in the formula for the greatest head became

$$\xi = \frac{(3.1416)\ (0.051)^4\ (981.0)\ (56.31 \times 1.02)\ (t)}{(8)\ (15.6)\ (5.0)}$$

ξ for any definite head may therefore be expressed as $\xi = K \times t$ so that the value for ξ at the different heads was $0.00192 \times t$,

$0.00155 \times t$ and $0.001177 \times t$. It was necessary only to multiply any one of these constants, depending on the head used, by the time in seconds required for 5.0 cc. of the casein solution to pass through the capillary to obtain the viscosity of each solution in poises. Since the solutions were viscous and not plastic, the determinations of the viscosity in poises at the three heads was a check on the accuracy of the determination. It is important to check by this method since small lumps in the solution in the capillary decrease the rate of flow and increase the value obtained for the viscosity.

Zoller (8) employing sodium caseinate solutions at pH 9.0, found that a 9.0 to 10.0 per cent caseinate solution is in the region of maximum sensitiveness to differences in reaction and to other factors such as salts, or ion effects. This experiment, as described by Zoller, has been repeated with substantially the same results. It was decided, therefore, to use 9.0 per cent solutions for the viscosity determinations and to adjust the reaction to a pH of approximately 9.1 to 9.2. Zoller also showed that a maximum viscosity occurs at about pH 9.10 to 9.25. Attempts by the authors to check these results were not successful. The viscosity somewhat above pH 9.1 to 9.2 increased, but not as rapidly as at less alkaline reactions.

It is known that casein solutions change in viscosity with time. Accordingly, a standard procedure was adopted for dissolving the casein in N/1 NaOH and making up the solution to approximately the concentration and reaction desired, allowing it to stand in the 10.0°C. cooler over night, adjusting the reaction and concentration to the exact point the next morning and making the measurements in the afternoon. The concentration of the solutions was based on the wet casein and the adjustment to the desired pH value was made in the beaker containing all of the solution. The antimony electrode was used. It was standardized with buffer solutions of known pH value and the pH of the sodium caseinate solutions as determined with the antimony electrode was found to check with the value given by the Bailey electrode method.

The reaction of the samples was determined by three methods:

1. Free acid. The method used for determining this property is empirical and was chosen because of its use in the casein industry. Ten grams were thoroughly mixed with 100 cc. of distilled water and the mixtures allowed to stand for one hour with occasional stirring. Fifty cubic centimeters of the supernatant liquid were then titrated with N/10 NaOH with phenolphthalein as the indicator. The value given for free acid is the number of cubic centimeters of alkali required to neutralize the acid in the 50.0 cc. of supernatant liquid.

TABLE 1

Properties of some acid caseins

SAMPLE NUMBER	WATER	ASH	FAT	FREE ACID	REACTION	ALKALI TO DISSOLVE TO pH 9.16 ± 0.02	VISCOSITY	STRENGTH	REMARKS
	per cent	*per cent*	*per cent*	*cc N/10 NaOH*	*pH*	*cc N/1 NaOH*	*centipoises*		
1	5.91	1.93	0.27	1.0	4.74	8.0	14.09	7	Coating mixture and solubility excellent
2	7.22	2.68	0.00	5.6	4.60	8.5	7.51	7	Coating mixture and solubility good
3	7.86	3.89	0.18	6.4	5.00	7.0	14.73	9	Coating mixture lumpy. Solution thick
4	8.03	3.76	0.18	6.2	5.03	7.0	12.22	11	Coating mixture slightly lumpy. Solution thick
5	8.35	3.90	2.70	5.0	5.20	6.0	13.88	9	Coating mixture slightly lumpy. Slowly soluble
6	7.48	5.11	0.45	7.8	5.14	6.2	8.57	12	Coating mixture and solution thick
7	6.23	3.63	0.18	13.0	4.32	10.2	14.36	11	Coating mixture smooth. Solubility fair
8	6.58	3.05	1.08	14.2	4.25	10.5	11.36	12	Coating mixture thick. Slowly soluble
9	6.30	3.39	0.09	15.2	4.35	9.6	11.66	11	Coating mixture and solubility poor
10	6.49	4.34	0.00	16.2	4.46	10.2	17.40	12	Coating mixture poor. Very insoluble

2. pH of casein. Two grams were well mixed with 2.0 cc. of distilled water and the pH determined by the quinhydrone

method in the same manner as was employed by Watson (9) for obtaining the pH of cheese.

3. The reasons for adjusting the reaction of the solutions employed for making viscosity determinations to pH of about 9.15 have been given. The cubic centimeters of N/1 NaOH required to do this depends upon the free acid in the casein and chiefly upon the pH at which the casein was put to press.

Solubility. The samples have been listed in the order of their solubilities as determined by inspection when making up the solutions for the viscosity and strength measurements.

The solutions of numbers 6, 7, and 10, which were made up for viscosity determinations, were milky in appearance and considerable difficulty was experienced in dissolving many fine white specks which looked like particles of insoluble casein.

Data on the samples of domestic casein obtained by the methods described are presented in table 1.

DISCUSSION

No definite standards have been established for the grading of casein. The domestic casein market would be better than it is if the manufacturers received a price based more on quality, as is the case in the marketing of other dairy products such as butter and dry skim milk.

The quality of casein is important. The manufacturer should, therefore, be concerned more about carrying out a process which will result in a product of good quality rather than in one which may give the largest yield.

It is not possible to judge the quality of casein from its general appearance. Dirt indicates lack of care in keeping equipment in good condition and may mean poor quality because of faulty manufacturing methods.

The properties of casein which is satisfactory for paper coating are not generally established. Strength is an important factor. If this were the only consideration, casein having a strength of 6 should sell for twice as much as casein having a strength of 12. A strong casein usually possesses other desirable properties.

The viscosity of the casein solution influences the manner in which the coating preparation will spread. Several of the values given in the table are low in this respect. An average figure for this property (based on unpublished data) for solutions tested as described above, is about 14.0 centipoises.

Much importance is attached to the borax solubility test as a criterion of quality. This test not only indicates the rate of solution, but also shows the presence and number of insoluble particles. It differentiates between caseins made at high and low temperatures. Cooked curd caseins tend to imbibe water and form a jelly.

The reaction of casein is influenced chiefly by the pH at which it was put to press and also by the amount of free acid present. If the whey is drained from the curd at the right degree of acidity and if the curd is washed effectively, the dry casein will have a pH of about 4.6, a low free acid value and also a low ash content.

The data in table 1, relating to free acid, show a wide variation. This acid must be neutralized before a coating preparation can be used and during the neutralizing process salts are formed which cause trouble, especially when satin white is used. If the casein is made so as to contain practically no free acid, less alkali will be needed and foaming is less liable to result.

The data in the table show properties of ten samples of commercial domestic casein. Only samples 1 and 2 can be classed as satisfactory. These samples are not presented as being representative of domestic casein, but it is not a good recommendation for the domestic product that so many of the samples obtained were so low in quality.

Most of the factors that affect the quality of one type of acid-precipitated casein affect all types. First of all sweet, clean, fat-free skim milk should be used. The keeping quality and strength of casein is decreased by the presence of fat and of course the return for the fat is much less than it would be if it were sold as cream or butter.

The precipitating temperature is important, especially where hydrochloric acid is used as the precipitating agent. When hydrochloric acid casein is made a temperature of 94 to 96°F. is necessary for best results. Below 94°, soft, fine curd forms that is hard to drain and wash, and which results in excessive mechanical loss in processing.

It is evident from analyses made by the Bureau of Dairy Industry of commercial samples of casein that the reaction of the whey at the time when the vat is dipped is not properly controlled. This should be at pH 4.1 or a titratable acidity of approximately 0.50 per cent measured as lactic acid with phenolphthalein as the indicator when the hydrochloric and sulphuric acid types are being made. The acid should be added slowly and the milk stirred constantly. Too fast stirring gives too fine a curd, and too slow stirring a lumpy curd. An excess of acid causes the formation of a gelatinous layer over the particles. This tends to make them stick together and injures their texture. Insufficient acid gives a milky, soft curd. In either case it is difficult to drain and wash the curd properly.

Casein occurs in milk in combination with calcium. The development of acidity (due either to fermentation or the addition of an acid) causes this calcium to be released and the casein to be precipitated. The acidity of the contents of the vat should be carried beyond the point at which, theoretically, the casein would be free of calcium. This is done to hasten the release of the calcium and to aid in the formation of a curd which will have a desirable texture.

The concentration of the acid used is usually too great. If sulphuric acid is diluted approximately 1:30, it will be found that a granular curd results from which the ash and acid can be much more easily washed, than if the acid is diluted 1:4 or 1:10.

The curd should be thoroughly drained and washed. The longer it is allowed to remain in contact with the whey the harder it is to wash it free of impurities. The curd still contains approximately 50 per cent moisture after it has been well pressed and is, therefore, readily subject to deterioration changes. The curd should therefore be dried as soon as possible after it is taken from the press.

So long as considerable water is present in the ground curd, it will not burn easily. If the curd is heated too quickly in the drier it will case harden. The first and last stages of the drying operation, then, should be watched more closely than the intermediate stage. So long as the final product contains less than 8 per cent moisture, it will not deteriorate quickly.

Insufficient attention has been given to the manufacture of casein. It should be carried out as a chemical process. Lack of realization of the importance of the manufacturing steps mentioned above is largely responsible for the quality of our domestic casein. It is not difficult to make high-grade casein, and when this is done the users will show their appreciation by paying a premium for it.

REFERENCES

(1) Sutermeister, E. 1929 Chemistry of pulp and paper making. John Wiley and Sons, New York, 2d Ed., p. 406.

(2) Shaw, R. H. 1918 Studies on technical casein: 3. Methods of analysis. Indus. and Engin. Chem., xii, 1168

(3) Sutermeister, E. 1927 Casein and its industrial applications. The Chemical Catalog Co., Inc., New York, p. 296.

(4) Cooke, R. D. 1924 The plastic properties of enamel slip Jour. Amer. Ceram. Soc., vii, 651.

(5) Ostwald, W., and Auerbach, R. 1925 Ueber die viskositat kolloider lösungen im Struktur-, Laminar- und Turbulenzgebiet. Zsigmondy Festschrift, Suppl. to Kolloid-Z., xxxvi, 252; 1926 Kolloid-Z., xxxviii, 261.

(6) Herschel, W. H., and Bulkley, R. 1927 Calibration of the Buret consistometer. Indus. and Engin. Chem., xix, 134.

(7) Bateman, G. M., and Sharp, P. F. 1928 Some observations on the consistency of cream and ice cream mixtures. Jour. Dairy Sci., xi, 380.

(8) Zoller, H. F. 1921 Casein viscosity studies. Jour. Gen. Physiol., iii, 635–651.

(9) Watson, P. D. 1927 Use of quinhydrone electrode for following changes of pH in Swiss cheese. Indus. and Engin. Chem., xix, 1272.

METHOD FOR HYDROGEN ION DETERMINATION OF BUTTER*

O. F. HUNZIKER, W. A. CORDES, AND B. H. NISSEN

Blue Valley Research Laboratories, Chicago, Illinois

Acidity has long been recognized as a factor of primary importance in influencing the keeping quality of butter. One phase of the problem of manufacturing salted butter of good keeping quality,—the prevention of the development of fishy and related off-flavors—is intimately related to the acidity of the cream at churning time and consequently the acidity of the butter. Unexplainable variations from the normal and expected results in isolated instances in storage of butter have cast some suspicion on the adequacy of the determination of acidity by titration methods. The true acidity as determined by hydrogen ion concentration may conceivably furnish a more reliable measure of the ability of butter to withstand at least this one type of deterioration.

The solution of this problem by means of hydrogen ion control of the cream at time of churning is a question that presents itself for future consideration. The work reported herein has to do with the hydrogen ion concentration of butter and suggests the possibility of the development of a test indicating the suitability of butter for cold storage. Preliminary studies and observations by the authors confirmed in a general way their earlier belief that there is a correlation between the pH of butter and its keeping quality. The results of our studies on the relation of pH value to keeping quality will appear in a later paper. The present paper is confined to a presentation of methods for determining the hydrogen ion concentration of butter that have been evolved from considerable experimentation. The establishment of these methods is the result of a study of the peculiarities of butter and the modification and precautions necessary to secure comparable and dependable results.

---

* Received for publication January 23, 1931.

DISCUSSION OF LITERATURE

Aside from the work of Parfitt (1), of Haglund, Platon, Waller and Soderberg (2), and of Lundstedt (3), the literature shows no reference to hydrogen ion determinations of butter and the relation of the hydrogen ion concentration to the keeping quality of butter.

Parfitt compared the pH value of butter before and after storage. His method consisted of melting the butter at 45°C., making a 1 to 10 dilution and comparing 1 cc. of this dilution in LaMotte (4) little dishes with LaMotte indicators. We have tried out carefully and extensively the method used by Parfitt and also the colorimetric method recommended by Sharp and McInerney (5) for milk. We have used dilutions of 1 to 1, 1 to 5, 1 to 10, 1 to 20, and 1 to 50 of both the serum of the butter and of the butter itself, employing the following indicators: brom cresol purple, brom thymol blue, methyl red, phenol red, chlor phenol red, and brom cresol green. As will be indicated later, neither the butter nor the butter serum can be diluted with water without affecting the pH reading. Whether it is possible to use a factor for butter, as is suggested by Sharp and McInerney for milk, we are not as yet prepared to say. Our results show marked irregularities caused by such dilutions of different types of butter. Likewise, the choice and behavior of indicators is accompanied by many inconsistencies in results. Our findings in regard to the colorimetric method are based on careful comparisons of colorimetric readings with electrometric determinations using both the quinhydrone and the hydrogen electrode.

We recognize the distinct advantages of the colorimetric method from the standpoint of simplicity of apparatus and procedure, and of economy of apparatus and butter samples. However, our work so far fails to show that this method is sufficiently reliable to justify its use for the pH determination of butter. We hope to show conclusively in a later paper if the colorimetric method can be made applicable to butter.

Haglund, Platon, Waller, and Soderberg studied the relation of the hydrogen ion concentration to the keeping quality of butter. Their pH determinations were made by the quinhydrone method, but the details of their procedure are lacking.

Lundstedt compared the hydrogen electrode with the quinhydrone electrode on various dairy products. This work came to our attention after preparing this paper for publication. His results show close agreement between the quinhydrone and the hydrogen electrode methods. Lundstedt found no difference between the pH of the undiluted and diluted portions of buttermilk, butter culture, and cream, and concluded that such slight dilutions in no way affected the measured potentials. This is somewhat at variance with our results and those of other investigators. As the result of his comparisons of the pH of butter between the quinhydrone and the hydrogen electrodes he concluded that no special salt or protein effects were apparent.

FACTORS INVOLVED IN THE PREPARATION OF THE BUTTER SAMPLE

Various procedures are in use for the preparation of products for pH determinations. Wherever possible, the use of the material in its original form is given preference. The peculiar make-up of butter, however, does not permit the determination of the hydrogen ion concentration on the whole butter in unmodified form as is the case with other dairy products, such as milk, cream, cheese, ice cream, condensed milk, etc. The butter fat comprising over 80 per cent of the composition of butter is not an electrolyte and refuses to establish connection between electrodes.

The preparation of the butter, therefore, becomes of fundamental importance. The method of preparation must be such as to avoid altering the pH of the butter, as far as possible, and the sample must be of adequate size and representative of the material to be examined.

In dairy products changes in reaction occur so quickly that the element of time may become very important. This is a factor with butter as it must be melted. The electrometric determination of pH on the butter direct and without dilution or removal of the fat is not feasible. This was conclusively established experimentally. No readings were obtained on butter unless the serum was allowed to separate out. These findings were further confirmed by using the method described by Watson (6) as satisfactory for cheese. In this method a small amount of the cheese and the required amount of quinhydrone powder are mixed together to form a paste which is placed in a tube containing the metal electrode. The same method tried with butter gave no readings at all, suggesting that in the butter-quinhydrone paste the fat constitutes the continuous phase. However, if the butter fat is allowed to separate, contact through the serum portion at the bottom is established and a reading is obtained. This method is not satisfactory, however, because of the fouling of the electrode with fat, and because of the difficulty of accomplishing a suitable mixture of the quinhydrone powder with the serum. In the presence of so much fat, the bulk of the quinhydrone powder remains in the fat and is lost to the serum where it is needed for the correct pH determination.

Because the fat is not an electrolyte its removal probably in no way influences the actual pH value of the butter. The fat may be regarded as an inert foreign substance, the presence or absence of which is of no consequence in the results of pH determinations of butter. In other words, the pH of butter is, in fact, the pH of the butter serum.

In arriving at these deductions we are cognizant of a possible fatty acid liberation resulting from partial decomposition of the fat. However, the water-soluble fatty acids, which would appear to be the only group of fatty acids capable of affecting the pH readings, should become a part of the serum when the butter is melted and, therefore, enter into the reaction of the butter serum.

APPARATUS FOR THE ELECTROMETRIC pH DETERMINATION

The apparatus used in the present procedure consists essentially of two parts, the voltage recording apparatus and the electrode assemblage. A Leeds and Northrup (7), Type K, potentiometer, a sensitive light ray galvanometer, and a two-volt storage battery with a Weston standard cell arranged for easy standardization of the storage battery voltage, constitutes the equipment for recording the minute voltages obtained. The standard cell was calibrated by the Bureau of Standards and has not suffered any noticeable change in voltage over a period of five years.

The electrode assemblage consists of three parts, the two electrodes and the vessel containing the liquid under examination. One of the electrodes is the calomel electrode, and the other is the hydrogen electrode or other electrode used in its place.

The exact type of calomel electrode is not so essential. It should be connected with a supply reservoir so that frequent flushing out with fresh KCl solution is possible. Only the very purest chemicals should be used in its preparation. Our experience has demonstrated that errors as great as 0.3 to 0.4 pH may result from the use of chemicals and electrodes that are not of extreme purity. About 1 ounce electrolytic mercurous chloride and about 8 ounces of redistilled mercury, such as prepared by the Eppley Laboratories, Newport, R. I., should be procured. With extreme care in its preparation the calomel electrode will last almost indefinitely and need never be changed.

The saturated KCl calomel electrode appears to be the most convenient although we have also used the N/10 KCl electrode. [illegible] only disadvantage in the use of the saturated KCl solution is its tendency to creep out at rubber connections and up over the edge of the salt bridge container. Such creeping may be readily prevented by applying a thin film of vaseline at the rubber connections and around the upper part of the salt bridge container immediately above the surface of the KCl solution.

To prevent undue crystallization of the KCl in the calomel cell and the KCl reservoir, the temperature of the surrounding air should not be permitted to drop much below about 20°C. To facilitate a rough control of the air temperature the authors placed their hydrogen ion apparatus in a cabinet of suitable size, and have been able to maintain the temperature of this cabinet between about 20° and 27°C. by the use of two nichrome "Sun Bowl" heater elements in series.

A small beaker serves admirably as the vessel for the solution to be tested. Some provision should be made to maintain the temperature of the solution under test precisely at the desired temperature, as even minor fluctuations in temperature markedly affect the voltage reading obtained. The authors provided a simple thermostatically controlled water bath in which the beaker containing the solution to be tested was immersed. A U-tube filled with 1.5 per cent agar in saturated KCl solution provided connection between the salt bridge and the test solution beaker. These U-tubes must be kept immersed in saturated KCl solution when not in use. They can be used repeatedly without any noticeable change in conductivity. The darkening effect of quinhydrone mixtures on the end of the immersed U-tube appears not to affect their accuracy.

Several different types of electrodes were used. These were the usual hydrogen electrodes, the quinhydrone electrode, and the antimony electrode. Of the various hydrogen electrodes used the simpler Hildebrand electrode with a No. 22 platinum wire protruding about $\frac{3}{16}$ inch from the glass tip appeared to be the most suitable. Difficulty in readily and thoroughly cleaning the plate type of electrode appeared to be a marked disadvantage here. To secure the best deposit of platinum black the authors used about $\frac{1}{2}$ ounce of a solution containing 3 grams of platinic chloride and 0.025 gram lead acetate per 100 cc. water. Two electrodes are preferably plated at the same time by connecting to the terminals of a two- or three-volt battery and reversing the current every fifteen to thirty seconds. Deposition for one to two minutes elapsed time usually gave the best results. The electrode must be thoroughly rinsed and kept in distilled water when not in use. Ordinary tank hydrogen gas was found sufficiently pure, no advantage being observed in the use of the customary gas washing chain. When in use the hydrogen gas should be passed through the electrode at a rate of about two bubbles per second.

Usually about twenty to thirty-five minutes was required for equilibrium to be reached in the case of the dairy products examined in the present experiment. The electrodes were periodically checked by the use of buffer solutions such as recommended by Clark (8), and the acetic acid sodium hydroxide buffer obtainable from Leeds and Northrup (7). The pH value for the hydrogen electrode was calculated by the use of the following formula, at 25°C.

$$\text{pH} = \frac{\text{voltage obtained} - 0.2458}{0.0591}$$

After considerable experimentation the quinhydrone method of Biilmann (9) with a gold spiral electrode was found to be the most suitable. A reversing switch, however, was required in the circuit since all voltages for a pH below about 7.7 are of an opposite sign as compared with the usual hydrogen electrode method. After each use the gold spiral electrode must be cleaned by rinsing in hot distilled water and from time to time in a 1 to 1 solution of ethyl alcohol and ethyl ether. The electrode must then be kept immersed in distilled water when not in use. The following formula was used for converting voltages obtained by the quinhydrone electrode into hydrogen ion readings, at 25°C.

$$\mathrm{pH} = \frac{0.6990 - \text{voltage obtained} - 0.2458}{0.0591}$$

As will be shown later, results obtained by the quinhydrone method on butter serum agree very well with the usual hydrogen electrode method. However, with buffer mixtures the quinhydrone method appears to give results a trifle higher, i.e., about 0.05 pH to 0.08 pH above that obtained by the hydrogen electrode.

The antimony electrode has been suggested by some students of hydrogen ion work (10). This electrode does not require hydrogen gas nor other material in the solution, such as quinhydrone. It consists merely of a piece of metallic antimony, about 1½ to 2 inches long and about ¼ inch in diameter with a copper wire soldered to it. This electrode is immersed directly in the liquid to be tested and is connected with the calomel cell in the usual way. It was believed that the antimony electrode might be used advantageously in certain phases of pH control, because of its simplicity. It suggests itself as applicable in the factory, as for instance, for pH determinations taken at the cream vat in connection with neutralization and ripening, and also as a rugged electrode for factory pH determinations on butter. For above reasons, we have compared the antimony electrode with the quinhydrone electrode, which latter had been selected for our present experiment. These comparisons, however, are not final and the tables given below are offered merely to show what results we obtained by the use of this electrode. The formula used for the antimony electrode at 25°C. is:

$$\text{pH} \quad \frac{\text{voltage obtained} - 0.050}{0.054}$$

Table 1 shows that very close agreement is possible between the antimony electrode and the quinhydrone on buffer solutions. On dairy products, however, the agreement observed so far was not quite so consistent. Incidentally it was noticed that the surface of the antimony tends to darken upon use, and can be brightened by the use of fine emery cloth or steel wool. Whether such discoloration may jeopardize the accuracy of the pH readings is as yet undetermined.

TABLE 1

Comparison of antimony and quinhydrone electrodes on buffer solutions and on milk and butter

MATERIAL TESTED	QUINHYDRONE ELECTRODE	ANTIMONY ELECTRODE
	pH	*pH*
Buffer solution:		
pH = 7.6	7 58	7 61
pH = 7.0	7 02	7 00
pH = 6.6	6 60	6 57
pH = 6.0	6 00	6 01
pH = 8.0	7 96	8 04
Sweet whole milk	6 62	6 85
Sour milk	4 29	4 20
Butter:		
Lot no. 1446	6 68	6 35
Lot no. 575 A.	6 35	6 10
Lot no. 575 B	6 53	6 77
Lot no. 575 C	6 88	6 95
Lot no. 556 A	4 71	4 79
Lot no. 556 B	4 99	4 95
Lot no. 556 C	5 27	5 25
Lot no. 863	6 31	6 36

EXPERIMENTAL

The method adopted in the present investigation was to determine the pH of the curd-serum, separated from the melted butter, by the quinhydrone method at 25°C. Our procedure was as follows:

Preparation of sample. Melt ½ to 1 pound of butter in a 1 quart sealed Mason jar, either by immersing the jar in hot water or by placing in a steam-water oven at a temperature of 75°C. Usually about one hour was required to completely melt 1 pound of butter and secure a satisfactory separation of the curd-serum from the fat. The butter so melted usually reached a temperature of approximately 55°C. No noticeable loss of moisture was observed by this method of melting. Before withdrawing any portion of the curd-serum shake the jar with a rotary motion so as to obtain a uniform mixture and to allow the serum to wash up into the fat. Now let the melted butter settle for a few minutes; then carefully remove as much as possible of the curd-serum with a 25 cc. pipette from below the fat. In the case of a 1 pound sample of butter from 70 to 85 cc. of curd-serum is usually obtained in this way. The first 25 cc. withdrawn is generally fairly clear serum, the remaining portion containing the curd. Mix all portions of curd-serum well for the pH determination.

Determination of pH by the quinhydrone method. Place about 50 mgm. of quinhydrone powder in a 100 cc. beaker, add to this about 1 cc. of the curd-serum and stir to a smooth paste. Then add the remainder of the 25 cc. curd-serum and mix with the paste until thoroughly homogeneous. Adjust the temperature of the mixture to exactly 25°C. and make the pH reading at this temperature, the beaker being immersed in a water bath thermostat.

Comparison of pH values by the quinhydrone and the hydrogen electrode methods. In order to confirm the accuracy of our results with the quinhydrone electrode, numerous check tests on the same samples of butter were made with the hydrogen electrode. The preparation of the samples was identical to that used for the quinhydrone determination except, of course, that no quinhydrone powder was used. This was in addition to comparisons made on standard buffer solutions and on milk and buttermilk on which the literature already contains several references to the close agreement possible between the two methods. Our purpose for making this comparison on the butter serum itself was to determine whether the quinhydrone powder reacted in any way with the constituents of the serum or interfered with the correctness of pH values of butter.

The pH values shown in table 2 cover determinations on the whole curd-serum of butter. These results show close agreement between the pH values obtained with the two electrodes. Differences were small and in no case exceeded 0.09 pH. Also the variations were in either direction, averaging 0.04 pH. The total average difference in pH values was only 0.01 pH. These comparisons thus serve to confirm the accuracy of the quinhydrone method for pH values within the usual range of hydrogen ion concentrations of butter.

TABLE 2

Comparison of quinhydrone and hydrogen electrodes on butter

SAMPLE OF BUTTER	QUINHYDRONE ELECTRODE	HYDROGEN ELECTRODE	DIFFERENCE	
			Quinhydrone higher	Hydrogen electrode higher
	pH	*pH*	*pH*	*pH*
1	6.13	6.12	+0.01	
2	6.07	6.04	+0.03	
3	6.18	6.13	+0.05	
4	6.41	6.36	+0.05	
5	6.73	6.71	+0.02	
6	5.74	5.65	+0.09	
7	5.54	5.58		−0.04
8	4.61	4.61	00	
9	6.09	6.04	+0.05	
10	5.90	5.94		−0.04
11	6.66	6.60	+0.06	
12	6.58	6.61		−0.03
13	6.73	6.77		−0.04
14	5.02	5.06		−0.04
15	5.48	5.52		−0.04
Average.	5.99	5.98	0.04	0.04

In considering these results it should be understood that they were not obtained on the butter as such, but upon the curd-serum of butter from which the fat had been mostly removed. It was impossible either with the Clark shaking electrode or the Hildebrand electrode to secure readings on undiluted melted butter even at temperatures high enough to keep the butter in perfectly melted condition (35°C. or above). However, with the quinhydrone method run at such high temperatures, direct determinations on butter were possible upon allowing the serum to settle sufficiently so as to provide connection between the electrodes. As an example of these experiences the following observations on the pH readings by the quinhydrone method of four variously prepared samples of the same butter are given:

	pH reading corrected to 25°C
Sample 1	
85 grams of butter melted in 100 cc. beaker at 35°C. 200 mgm. of quinhydrone added and mixture well stirred. pH reading on this while stirring continued was	None
Stirring was then discontinued and curd-serum mixture was allowed to settle. In this condition the following reading was obtained	5 86
Sample 2	
85 grams of butter melted as above and fat poured off from serum as completely as possible. Quinhydrone powder added to serum remaining in beaker; pH reading was	5 80
Sample 3	
130 grams of melted butter centrifuged in a separatory funnel at 35°C. and curd-serum removed. pH reading on curd-serum was	5 85
This method involves the added difficulty of completely reclaiming the curd from the funnel.	
Sample 4	
300 grams of butter melted in a sealed Mason jar. Curd-serum pipetted from bottom of jar, as described under "Preparation of sample." pH reading on curd-serum at 25°C. was	5 83

The above examples show that pH readings cannot be made conveniently on the whole butter except when the curd-serum is permitted to separate and in such cases only when run at 35°C. to prevent the fat from solidifying. When the curd-serum alone is used for the sample, instead of the whole butter, the readings can all be made at 25°C. It was to eliminate these various difficulties of technique encountered when attempting to use samples of the whole butter that we standardized on the curd-serum alone as the most practicable and reliable method for the determination of the pH of butter.

FACTORS INFLUENCING ACCURACY OF METHOD

In the preparation and use of the butter sample for pH determinations, as described in previous paragraphs, certain factors were encountered which required further study. The more important of these are the temperature to which the butter sample is heated for melting and for satisfactory curd-serum separation, the relative effect on pH readings of the curd and serum part of the curd-serum mixture, and the effect of dilution of the sample.

TABLE 3

Effect of temperature used in preparation of sample on pH reading

TREATMENT OF SAMPLE	CHURNING NUMBER		
	1	2	3
	pH	*pH*	*pH*
Butter melted at 75°C.	6 04	7 25	7 32
Buttermilk not heated, fresh	5 95	6 78	6 86
Buttermilk not heated, held at 5°C. for 1 hour		6 75	6 84
Buttermilk not heated, held at 5°C. for 1½ hours		6 73	6 81
Buttermilk not heated, held at 5°C. for 2½ hours	5 90	6 68	6 79
Buttermilk not heated, held at 5°C. for 7 hours	5 85		
Buttermilk heated in water oven at 75°C. for 1 hour		6 53	6 81
Buttermilk heated in water oven at 75°C. for 1½ hours		6 41	6 76
Buttermilk heated in water oven at 75°C. for 2½ hours	5 69	6 42	6 76
Buttermilk heated in water oven at 75°C. for 7 hours	5 63		

Effect of temperature to which butter is heated. It has been shown by the work of Duncombe (11), Cosmovici (12), Whittier and Benton (13), and Sharp and McInerney (5), that the heating of milk causes a slight increase in the hydrogen ion concentration as shown by a slight drop in the pH reading. These findings are important because of their possible relation to the accuracy of pH determinations on butter, for in order to secure a satisfactory separation of the curd-serum, butter must be heated to temperatures considerably in excess of the normal temperature (25°C.) at which the readings are made. In the present experiment, as previously stated, the butter was held at 75°C. for approximately one hour's time and the temperature of the melted butter was usually about 55°C. In order to determine the possible effect of such temperature and time exposure, buttermilk was heated to various temperatures and the pH was determined on the samples so heated as well as on the butter from the same churning, as shown in table 3.

TABLE 4

pH values of butter and buttermilk

SAMPLE NUMBER	BUTTERMILK	BUTTER, I E CURD-SERUM OF BUTTER	AVERAGE	
			Buttermilk	Butter
	pH	*pH*	*pH*	*pH*
1472	5 94	6 03	6 02 Average for churnings in which the pH of the buttermilk was below 6.50	6 05
1473	5 94	6 03		
1478	5 84	6 00		
1479	5 72	5 83		
308	6 35	6 31		
309	6 37	6 29		
311	6 34	6 16		
623	4 76	4 83		
328	6 47	6 43		
329	6 40	6 48		
1017	6 09	6 21		
1008	6 66	7 00	6 76 Average for churnings in which the pH of the buttermilk was above 6.50	7 11
1011	6 73	7 13		
1023	6 82	7 06		
1025	6 73	6 88		
946	6 78	7 25		
108	6 86	7 32		

The results given in table 3 indicate that the heating of buttermilk does depress the pH reading slightly. This is in harmony with the results on milk reported by other investigators. The buttermilk temperature rose to from 52° to 55°C. during the one hour heating period and the reading dropped approximately 0.2 pH. It appears reasonable to assume, therefore, that the same treatment of the butter sample, in order to obtain the curd-serum separation, may have a similar depressing effect on the pH value of butter.

Additional information on the effect of melting of the butter sample on the pH reading was obtained by melting a sample in running hot water in 15 minutes time. The pH of the curd-serum of this sample was 6.48 while three other samples of the same butter melted in the usual way gave readings of 6.41, 6.38, and 6.39. This would indicate that the standard method of heating for one hour does have a slight depressing effect on the pH.

This slight decrease in pH suggests the possibility of bacterial action with its resultant increase in acidity. However, plate counts on litmus-lactose agar made on samples of both butter and buttermilk before and after heating showed a definite, significant reduction in number of colonies as a result of the heating. Furthermore, plates incubated at 62°C. showed no growth, indicating the absence of thermophiles. A modification of the methylene blue test on the same sample of buttermilk, before and after heating, showed a definitely longer reduction time for the sample after heating, e.g., ten minutes as compared to two hours. These results indicate not only that bacterial growth is not involved but rather point toward a destruction of bacteria during the heating of the sample; hence the decrease in pH must be due to factors other than bacterial action.

Comparisons of the pH of buttermilk and butter from the same churning show a higher pH reading for the butter, notwithstanding any depressing effect of the heating of the sample. This is shown in table 4.

The pH values in table 4 refer in each case to butter and buttermilk from the same churning, and show that the differences in pH vary somewhat with the range of pH. Churnings in which the buttermilk has a relatively high pH yielded butter with a greater difference between butter and buttermilk than churnings in which the pH of the buttermilk was not so high or was low. The eleven churnings showing a buttermilk pH below 6.5 averaged a difference between buttermilk and butter of only 0.03 pH, the buttermilk having a pH of 6.02 and the butter a pH of 6.05.

The six churnings showing a buttermilk pH above 6.5 averaged a difference between butter and buttermilk of 0.35, the buttermilk averaging 6.76 and the butter 7.11 pH.

The figures in table 4 further support the results indicated in table 3, that our method of preparing the butter sample by melting in a water oven at 75°C. has only a slight effect, if any, on the pH value of the butter. In these experiments, the buttermilk sample was not heated while the butter sample was heated in accordance with the method already described. Yet the 17 churnings show an average of 0.14 pH higher for the butter than for the buttermilk. Assuming that the butter and the buttermilk should have the same pH value, which is not as yet proven, the above would indicate that, if the heating of the butter sample caused any decrease in pH, such decrease must have been slight, as it appears to be more than offset by other factors not as yet determined. An interesting relationship is further shown in table 5, in which the pH values of cream, buttermilk, butter granules before washing and salting, and the finished butter are compared.

TABLE 5

pH values of cream, buttermilk, unwashed butter granules, and finished butter of the same churnings

CHURNING NUMBER	CREAM	BUTTERMILK	UNWASHED BUTTER GRANULES	FINISHED BUTTER
	pH	*pH*	*pH*	*pH*
1008	6 71	6 66	6 96	7.00
1011	6 73	6 73	7 16	7 13
1017	6 15	6 09	6 07	6 21
1023	6 87	6 82	7 10	7 06
1025	6 75	6 73	7 05	6 88
1026*	6 75	6 68	6 88	6 48
Averages.	6 66	6 62	6 87	6 86

* Omitted from average, 4 gallons of culture worked into 900 pounds of butter with the salt.

The figures in table 5 show very close agreement of pH values between cream and buttermilk. Where there is a difference the pH of the cream is slightly higher than that of the buttermilk, the maximum difference is 0.07 pH and the average 0.04 pH. Omitting from consideration churn lot 1026 because culture was added to the butter, the finished butter averaged only 0.01 pH lower than the unwashed granules. The unwashed butter granules averaged 0.25 pH higher than the buttermilk, suggesting that there may be an uneven distribution of the factors affecting the pH readings when such sour, neutralized cream is churned. The tendency for the curd-serum to show a higher pH than the buttermilk has masked any slight depressing action of the heating of the sample that might have occurred. The working of culture into the butter lowered the pH of the finished butter 0.4 below that of the unwashed butter granules.

In considering these results attention should be called to the fact that all of these churnings were made from sour, neutralized cream. It is conceivable that the presence of a neutralizer may have some effect on the pH readings in both buttermilk and butter that would be absent in the case of buttermilk and butter made from sweet cream or from ripened cream not neutralized. The effect of neutralization on the relation of pH of buttermilk to pH of butter will be shown in a later paper.

Effect of curd on the pH reading. In preparing the butter sample every effort was made to accomplish as complete a separation of the curd-serum mixture from the fat as possible. Centrifuging, dilution and washing with water, and removal of the curd-serum from the melted butter sample by means of a pipette were compared.

The centrifuging of the melted butter was conducted both in 250 cc. bottles and in 250 cc. separatory funnels. This method proved unsatisfactory because a layer of curd which was difficult to remove packed solidly against the bottom of the flask and against the stopcock of the separatory funnel. Furthermore, below the fat there was a layer of mixed fat and curd which made complete separation of the fat and satisfactory reclaiming of the curd difficult.

In the method by dilution a definite amount of freshly boiled distilled water was added to the melted butter and the sample shaken to wash out the curd-serum. In other cases water was used to wash the fat after withdrawing the curd-serum. It will be shown later that dilution of the butter serum is not advisable because of its disturbing effect on the accuracy of the pH reading.

Because of the above findings, both centrifuging and dilution were abandoned and the simple method of merely pipetting off the total amount of curd-serum from below the fat layer of the melted butter sample was adopted. This method provides more complete fat separation than is possible by means of centrifuging. However, in some cases, particularly in the presence of gassy curd, some particles of the curd refuse to settle into the curd-serum mixture and may be lost to the mixed sample of curd-serum. These facts emphasize the importance of definite knowledge regarding the part which the curd plays in the pH determination of butter. It was considered advisable, therefore, to conduct further tests to determine the relation between the aqueous part

TABLE 6

pH in aqueous portion and in curdy portion of buttermilk

SAMPLE NUMBER	TEMPERATURE OF SEPARATION	pH IN BUTTERMILK		DIFFERENCE + CURDY PORTION HIGHER − AQUEOUS PORTION HIGHER
		Aqueous portion	Curdy portion	
	°C			
1	63	5 55	5 67	+0 12
2	115	5 59	5 63	+0 04
3	Not heated	5 85	5 81	−0 04
4	63	6 41	6 58	+0 17
5	50*	5 66	5 85	+0 19
6	115	6 79	6 93	+0 14
7	115	6 23	6 45	+0 22
8	115	7 21	7 59	+0 38
9	115	6 89	7 14	+0 25
10	50	6 99	7 00	+0 01
11	55	6 99	7 01	+0 02
12	63	6 91	7 14	+0 23
13	55	6 46	6 47	+0 01
Averages		6 42	6 56	+0 14

* Held for six hours.

of the curd-serum and the curdy portion. These results are shown in tables 6 and 7.

The figures shown in table 6 refer to the aqueous and curdy portions of buttermilk which were separated as completely as possible by repeated centrifuging. The results average 0.14 pH higher for the curdy portion. However, the individual differences between the two portions vary from 0.38 higher for the curdy portion to 0.04 pH higher for the aqueous portion. These variations are very probably attributable to difference in the relative completeness of separation of the curd from the serum. In order to secure any separation it was necessary to heat the buttermilk. This was done at temperatures varying from 50° to 115°C. for one-half to two and one-half hours. At the higher temperatures the separation was most nearly complete so that in these cases the difference in pH between the aqueous and the curdy portions should be greatest, provided that the curd in-

TABLE 7

pH of the curd-serum mixture of butter, its aqueous portion, and its curdy portion

SAMPLE NUMBER	CURD-SERUM MIXTURE	AQUEOUS PORTION	CURDY PORTION	DIFFERENCE—EXCESS OF pH OF CURDY PORTION OVER AQUEOUS PORTION
	pH	*pH*	*pH*	*pH*
1	6.20	6.06	6.27	0.21
2	7.25	6.90	7.22	0.32
3	7.31	7.11	7.37	0.26
4	6.07	5.88	5.96	0.12
5	6.39	6.25	6.41	0.16
6	7.25	7.19	7.24	0.06
7		6.14	6.30	0.16
8		5.88	5.96	0.12
9		6.14	6.30	0.16
10		6.03	6.15	0.12
11		6.07	6.09	0.02
Averages for samples 1 to 6...	6.74	6.56	6.74	0.18
Averages for all samples.........	—	6.33	6.48	0.15

fluences the pH. This was in fact the case wherever satisfactory separation was possible. These results indicate that the curdy portion does yield a slightly higher pH than the aqueous portion of the buttermilk.

The method of separation of the aqueous and curdy portions from butter was as follows: One pound of butter was melted in the usual way but without shaking at end of heating. With a pipette 25 cc. of a fairly clear aqueous serum could be removed. This represented the aqueous portion. More of the aqueous material was then pipetted out and rejected until curdy material began to enter the pipette. At this point 25 cc. of the thick curdy layer was removed. This represented the curdy portion.

The results on the pH of the aqueous and the curdy portions of butter, as given in table 7, further demonstrate that absence of the curd tends to lower the pH reading. In the eleven churnings studied the differences averaged 0.15 pH and in each individual comparison it was the curdy portion that yielded the higher pH reading. In those comparisons in which the pH of the curd-serum mixture also was determined, the results show close agreement between the whole curd-serum mixture and the curdy portion, while the aqueous portion yielded a consistently lower pH. It is obvious from the above description that, while the aqueous portion used was practically free from curdy material, the curdy portion used contained considerable liquid along with the curd. Had a more complete separation of the curd portion been possible, the difference in pH between the curd portion and the aqueous portion might have been greater.

In comparing the hydrogen ion concentration or true acidities with titratable acidities of butter serums it was observed that in the case of the titratable acidity the serum acid was lower than the curd acid while in the case of true acidity the opposite was the case. Similar findings have been recorded by Whittier and Benton (13) in the case of milk. To further substantiate the results secured by the quinhydrone electrode some of the samples of curdy and serum material were run both by the quinhydrone and hydrogen electrode methods. Exactly the same difference in pH between the curdy portion and the serum portion was shown by the hydrogen electrode method as by the quinhydrone procedure.

These results, both with buttermilk and with butter, leave little doubt that the curd does have a slight but unmistakable effect on the pH reading. They show that the curd is an integral part of the curd-serum mixture and that this mixture gives a true reading only if all the curd is included in it. This emphasizes the advisability of using as large a sample of butter as possible (about 1 pound) so as to obtain a reasonably large amount of curd-serum mixture.

TABLE 8

pH of whole milk before and after dilution

SAMPLE NUMBER	ACIDITY OF WHOLE MILK	ORIGINAL WHOLE MILK	RATE OF DILUTION		
			1 to 5	1 to 10	1 to 20
	per cent	*pH*	*pH*	*pH*	*pH*
1	0 16	6 58		6 92	7 02
2	0 20	6 45		6 67	6 91
3	0 16	6 63		7.00	7 14
4	0 20	6 25		6 85	6 99
5	0 16	6 61		7 00	7 07
6	0 17	6 58		7 00	7 08
7	0 17	6 50		6 91	7 00
8	0 16	6 60		7 00	7.08
9	0 18	6 59		7 01	7 09
10*	0 16	6 83	7 08	7 18	
Averages	0.173	6.532		6 93	7 04
Increase by dilution			+0 25	+0 40	+0 51

* No. 10 is not included in the averages but is given to show the relation of the 1 to 5 dilution.

Effect of dilution of sample on pH value. It would be a distinct advantage to be able to dilute the butter sample, so as to reduce the amount of butter required and to make possible the washing of the fat for more complete reclaiming of the curd and acid. If butter serum were well buffered it should be possible to dilute it with several volumes of water without affecting its pH value at all. Such was found to be the case with peptone water and also with nutrient bouillon as shown by Sharp and McInerney (5). These liquids were diluted as much as 20 times without changing their pH value. However, in the case of milk the above investigators and also Taylor (14) show that the pH value rises with the dilution of the milk. Furthermore, Sharp and McInerney consider that within certain pH ranges the rise was uniform, and upon the strength of their findings they developed certain dilution factors for use in colorimetric determinations of the pH on milk. The correction factors adopted ranged from 0.15 pH for milk having a pH around 4.4, to 0.54 pH for milk having a pH range of 6.2 and above. The above data suggested similar experimentation with butter. This study was preceded by dilution experiments on milk for confirming this effect on the pH electrometrically. Table 8 summarizes these results.

TABLE 9

pH of curd-serum mixture from butter undiluted and diluted

CHURNING NUMBER	UNDILUTED CURD-SERUM MIXTURE	DILUTION 1 to 1		DILUTION 1 TO 5		DILUTION 1 to 10		DILUTION 1 TO 50	
		pH value	Increase	pH value	Increase	pH value	Increase	pH value	Increase
	pH		*pH*		*pH*		*pH*		*pH*
711	6 18			6 68	0 50	6 71	0 53	6 85	0 67
1292	6 13					6 73	0 60		
1624	5 68					6 22	0 54		
2078	6 01					6 56	0 55		
2243	6 29					6 75	0 46		
556	4 71			4 99	0 28	5 27	0 56		
1	6 25					6 63	0 38		
2	6 07					6 66	0 59		
1478	5 98	6 28	0 30						
1472	5 12	5 21	0 09						
1049	6 30					6 90	0 60		
1810	5 62					5 75	0 13		
1042	6 22					6 56	0 34		
1032	6 41					6 73	0 32		
2312	5.78					6 29	0 51		
3170	6 09					6 43	0 34		
Average pH increase by dilution.			0 195		0 39		0 46		0 67

The results given in table 8 agree in general with those of the literature cited. Dilution of the milk increased its pH value. The increase progressed in the direction of the dilutions. The pH rose 0.25, 0.40, and 0.51, respectively, for dilutions of 1:5, 1:10, and 1:20.

In the case of butter, there are two possibilities for dilution, namely, dilution of the whole butter and dilution of the curd-serum of the butter after separation and removal from the melted butter. pH values were obtained on dilutions of both the curd-serum mixture and the whole butter, as indicated in tables 9 and 10.

TABLE 10

pH of undiluted curd-serum mixture and of diluted butter

CHURNING NUMBER	UNDILUTED CURD-SERUM MIXTURE	BUTTER DILUTION 1 TO 4		BUTTER DILUTION 1 TO 5		BUTTER DILUTION 1 TO 10	
		pH value	Increase	pH value	Increase	pH value	Increase
	pH		*pH*		*pH*		*pH*
XFM	5.37	5.65	0.28				
1023	7.10	7.60	0.50				
1690	6.33	6.86	0.53				
1678	6.45	6.85	0.40				
956	6.18	6.73	0.55				
1017	6.07	6.49	0.42				
1011	7.16	7.30	0.14				
1446	6.22			6.68	0.46		
1292	6.13			6.58	0.45		
1297	5.94			6.30	0.36		
575	6.32			6.53	0.21	6.86	0.54
1038	6.00					6.56	0.56
2288	5.93					6.57	0.64
1624	5.68					6.27	0.59
2078	6.01					6.66	0.65
2	6.07					6.61	0.54
Average pH increase by dilution.			0.40		0.37		0.58

Tables 9 and 10 show that, similarly as in the case of milk, dilution increases the pH value of butter, both when the curd-serum mixture of butter was diluted and when the whole butter itself was diluted. The increase in pH was greatest for the highest dilutions, but it lacked in uniformity and it was not directly proportional to the rate of dilution.

On the basis of the present limited data, it appears evident that the increase in pH on the diluted sample is not sufficiently consistent for accurate pH determinations. Further work may, however, develop that a certain dilution of the butter may justify the use of a factor for the pH of butter, in a similar way as Sharp and McInerney have suggested for milk for colorimetric determinations. Our results show that without such a factor the dilution of butter yields erroneous pH readings.

TABLE 11

pH values of butter from different parts of the same churning

NUMBER OF PORTIONS FROM CHURNINGS	CHURNING NUMBERS										
	1422	946	1201	321	1124	1446	180	1297	1292	711	L B
1	6 14	7 25	6 35	6 38	6 69	6 22	7 32	5 97	6 13	6 18	6 07
2	6 14	7 26	6 25	6 41	6 60	6 20	7 31	5 94	6 07	6 20	5 96
3	5 98	7 22	6 37	6 38	6 70	6 22	7 24				
4	5 99	7 22	6 35	6 39	6 73						
5	6 09	7 23									
6	6 09										
7	6 07										
8	6 03										
Average pH values .. .	6 07	7 24	6 33	6 39	6 68	6 21	7 26	5 96	6 10	6 19	6 02
Maximum deviation from average	0 09	0 02	0 08	0 02	0 08	0 01	0 06	0 02	0 03	0 01	0 06
Maximum differences between portions	0 16	0 04	0 12	0 03	0 13	0 02	0 08	0 03	0 06	0 02	0 11

Uniformity of pH in different portions of the same churning. In obtaining samples of butter for pH determinations the representativeness of the sample taken becomes an item of consideration. This is suggested by the fact that butter is not a product of perfectly uniform composition, hence variations may occur in the hydrogen ion concentration. If such were the case the pH determinations on a single sample of butter would be subject to justifiable criticism. Check tests were, therefore, made on two or more portions of the same churning as shown in table 11.

The figures given in table 11 demonstrate that different portions of butter from the same churnings in most cases do show minor variations in pH. These variations, however, are not sufficiently great to interfere with the value of the pH determinations made on single packages only.

Other factors that may influence the accuracy of pH determinations of butter. One of the factors meriting consideration is the high concentration of the salt in the serum, the serum of average salted butter made in this country being practically a saturated solution of sodium chloride. It is pertinent to suspect the possibility of a "salt error" here, as is sometimes encountered in colorimetric work. Although our present experiment made no attempt to determine this, we have reason to assume that the salt does not cause any material deviation in pH value. Our assumption is based in part at least on the results shown in table 5 and also on additional but unpublished work on salted and unsalted butter made from the same lots of butter granules. In considering the above we are aware of the work done by Sorensen et al. (15) in which they show that the pH by the quinhydrone method on N/100 HCl solutions is depressed slightly by the addition of sodium chloride. For a salt concentration similar to that of the serum of average American salted butter, this depression would appear to be about 0.1 to 0.15 pH.

In addition it is believed that the protein may have an effect on the results obtained by the quinhydrone electrode. Linderström-Lang (16) shows that in the presence of about 0.7 per cent casein in N/20 HCl the quinhydrone electrode differs by about 0.005 pH from the hydrogen electrode. Our results on the curd-serum mixture, table 2, show close agreement between the two electrodes. Whether or not these two factors, i.e., the salt and the protein errors are of similar magnitude in butter, and whether they function in the same manner and direction, or opposite to each other is uncertain.

Another factor has to do with the effect of the fat. The fat may be considered to influence the pH readings in two ways. First, the removal of so large a portion of an ingredient of a product in such a crude way may be open to question. It might rob the portion actually examined, i.e., the curd-serum mixture, of a sufficient amount of constituents that influence the test, such as acids, curd, and salts, to interfere with the accuracy of the final results. To what extent, if any, they may be a factor, is a question for further study. In the second place, unless special precautions are taken, small portions of fat escape into the curd-serum portion and might thus interfere with the accuracy of the pH reading. Preliminary experimentation in the development of the method of preparation of the butter sample, however, indicates that such small entrained amounts of fat have no noticeable effect on the pH reading.

SUMMARY AND CONCLUSIONS

1. The work herein reported constitutes a study of the factors involved in the electrometric determination of the hydrogen ion concentration of butter.
2. A simple procedure for the preparation of the butter sample and for the application of the quinhydrone electrode to the hydrogen ion determination is described. Its use as a standard method in similar studies is suggested.
3. It is shown that on account of the non-conductive property of butter fat the curd-serum portion must be used in the pH determination.
4. The quinhydrone electrode was found to give essentially the same results on butter as the standard hydrogen electrode.
5. Dilution of the butter or of the curd-serum mixture caused marked changes in the pH values obtained, leading to erroneous results.
6. Removal of the curd from the curd-serum mixture tended to lower the pH value slightly.

REFERENCES

(1) Parfitt, E. H. 1927. The hydrogen ion concentration of cold storage butter. The World's Butter Review, i, no. 3, 11.

(2) Haglund, E., Platon, B., Waller, E., and Soderberg, E. 1929. The effect of the method of preparation on the keeping qualities of butter. Medd. Centralanstalt forsoksvasendet jordbruks, no. 347, Mejeriavdel no. 37.

(3) LUNDSTEDT, E. 1930. A comparison of the measurements by the hydrogen and the quinhydrone electrodes in certain dairy products. M.S. thesis, Iowa State College (Unpublished).

(4) LaMotte Chemical Products Co. 1929. The ABC of hydrogen ion control. Fifth edition, p. 96.

(5) SHARP, P. F., AND McINERNEY, T. J. 1926. The colorimetric determination of the hydrogen ion concentration of milk, whey, and cream. Jour. Biol. Chem., lxx, no. 3, 729.

(6) WATSON, P. D. 1929. The relation of the hydrogen ion concentration to the texture of Emmenthal or Swiss Cheese. Jour. Dairy Sci., xii, no. 4, 289.

(7) Leeds and Northrup Co. 1930. Notes on hydrogen ion measurements, Note Book No. 3.

(8) CLARK, W. M. 1928. The determination of hydrogen ions. Third edition.

(9) BIILMANN, E. 1924. On the measurement of hydrogen ion concentrations in soil by means of the quinhydrone electrode. Jour. Agr. Sci., xiv part II, 232.

(10) SNYDER, E. F. 1928. The application of the antimony electrode to the determination of the pH values of soils. Soil Sci., xxvi, no. 2, 107.

(11) DUNCOMBE, E. 1924. The influence of certain factors on the hydrogen ion concentration of milk. Jour. Dairy Sci., vii, no. 3, 245.

(12) COSMOVICI, N. L. 1925. Compt. rend. soc. biol., xcii, 73.

(13) WHITTIER, E. O., AND BENTON, A. G. 1926. The effect of heating on the hydrogen ion concentration and on the titratable acidity of milk. Jour. Dairy Sci., ix, 481.

(14) TAYLOR, H. B. 1913. Jour. and Proc. Roy. Soc., N.S. Wales, xlvii, 174.

(15) SORENSEN, S. P. L., SORENSEN, M., AND LINDERSTROM-LANG, K. 1921. About the salt error with the quinhydrone electrode. Comptes Rendus trav. lab. Carlsberg, xiv, no. 14.

(16) LINDERSTRÖM-LANG, K. 1926. Measurements of quinhydrone electrode. Comptes Rendus trav. lab. Carlsberg, xvi, no. 3.

THE HEMOGLOBIN AND TOTAL PHOSPHORUS IN THE BLOOD OF COWS AND BULLS

C. M. McCAY

Animal Nutrition Laboratory, Cornell University, Ithaca, New York

Much recent work especially in Germany has been concerned with various anatomical and blood analytical measurements upon milking cows. The physical measurements have been concerned with the size of various portions of the body. The chemical measurements have dealt with such blood constituents as total solids and hemoglobin. Kronacher and his students have reported many such studies (1). In their earlier papers they found a correlation between the fat of the milk and the total solids of the blood in lactating goats. In their later papers (2) they seem to get no correlations between either the physical measurements or the blood constituents and the productive capacities of cows.

The physical measurements of Kronacher (1) included the height at withers, the depth, width and girth of the breast, the hip width, the width and length of pelvis and the rib angle.

The blood constituents and properties that were considered by Kronacher are hemoglobin, dry substance, blood viscosity, plasma viscosity, serum viscosity, erythrocyte sinking rate and alkaline reserve. In both papers Kronacher has called attention to the high positive correlation between the hemoglobin and the total dry substance of the blood.

Since the blood data of Kronacher were not convincing, we decided to run monthly hemoglobin determinations upon the blood of the animals in the Cornell University herd for a period of one year. One thousand and seventy-three analyses were made by the method of Cohen and Smith (3) upon female animals. These included sixty-nine mature cows and twenty-one other younger animals. The mean of all hemoglobin determinations upon female animals was 10.9 ± 0.86 grams of hemoglobin per 100 cc. of blood. During the course of the same year seventy determinations were made upon six mature bulls of four different breeds. The mean value for these animals was 12.8 ± 0.8 grams of hemoglobin per 100 cc. of blood. There is a significant difference between the hemoglobin values of the male and female animals. This is the reverse of the findings of Anderson (4), who found females had the higher hemoglobin values. It accords with long established values upon human blood which show that the normal male has more erythrocytes and more hemoglobin per unit volume of blood than the female. In most animal species the male has more hemoglobin per unit volume of blood than the female. Schulz and Krüger (5) have pointed out some exceptions to this rule, however.

Our cows studied included thirty-eight Holsteins, eight Ayrshires, ten Guernseys, and thirteen Jerseys. The mean for each of these breeds is the same within the experimental error. There is no correlation between breed and the hemoglobin level. The following are the mean values for the cows of various breeds in grams of hemoglobin per 100 cc. of blood:

Holstein	10 6 ±0.47
Ayrshire	10 7 ±0 61
Guernsey	10 4 ±0 44
Jersey	10 5 ±0 32

Correlation factors were worked out to determine any relations that might exist between the hemoglobin of the blood and the following variables: the monthly milk production, the monthly fat production, the prolongation of the lactation period, the gestation period. We found no correlation between the hemoglobin and any of these variables. Since some of our age groups were very limited in number, there may have been an age relation here that we could not measure. Since the hemoglobin is known to bear a close relation to the total solids of the blood, we are probably justified in assuming the same result would have been obtained if we had measured total solids. Both total solids and hemoglobin measurements are crude in comparison to the more delicate chemical analytical methods for blood constituents. Our data do not imply that there is no relation between the chemical constitution of the blood and the production or physiological state of the animal.

An analysis of our data to detect seasonal trends in the hemoglobin level indicated a slight rise during the summer months. Reports from various laboratories have shown increases in the hemoglobin of the blood of lactating cows during the summer months. A close scrutiny of these reports shows that part of them can be neglected either because very few animals were involved in the study or because questionable analytical methods were employed. Hemoglobin has frequently been determined by the use of such standards as the Newcomber glass slide. If this slide is not carefully calibrated the data are usually questionable. In the past six years the author has had occasion to check three of these slides. Only one of these was found to be reasonably accurate.

Since the rise in hemoglobin we found during the summer months was within the range of questionable significance, we decided to repeat a portion of the work. Nearly two years elapsed between our first experiment.and the repetition. In the meantime the composition of the herd previously studied had been altered and a new herd was also available. These new conditions permitted us to consider several variables. The new herd consisting of thirty-nine Holstein cows, was upon the protein experiments being conducted by E. S. Savage and E. S. Harrison. At the time of the first hemoglobin determinations these cows were in their second lactation periods upon the experimental rations. All cows in this herd were fed corn silage as well as clover and timothy hay. One-third received a grain mixture containing 16 per cent protein, another 20 per cent while the last was fed a 24 per cent mixture. Each group of thirteen cows had been upon the experimental diets for approximately eighteen months at the time of our first analyses. This herd gave us an opportunity of detecting any influence these protein-level differences might have upon the hemoglobin.

This was of special interest since we felt the differences detected by others in the summer and winter and hemoglobin might be a measure of the winter management. With inadequate housing or poor feeding during the winter months one might expect a tendency toward anemia in the spring. In these experiments with different protein intakes one might look for a reflection in the blood proteins in case the minimum was low enough to create a deficiency. Since this herd was allowed sunshine and fresh air in a lot with practically no pasture it afforded an opportunity to study these variables. At the same time another herd of thirty-five milking cows which was turned out to pasture shortly after the middle of May was available.

Kronacher (2) has found the dry matter of the blood of cows increases during the summer by about ten per cent. Van Gelder (6) found that the hemoglobin and red cells of the Swiss mountain cattle increases in the summer. In our second study we sampled the blood of all animals in both experimental herds at the middle of May just before the period of turning them out to the lot or pasture respectively. A second series of samples was taken the middle of July after a two-months period of pasture or dry-lot exposure. Not only hemoglobin, but also iron and total phosphorus determinations were made upon all blood samples. Iron was included as a check upon our hemoglobin methods. In our data we have expressed iron as such. We believe this is preferable to conversion to hemoglobin by the use of a questionable factor.

Several refinements such as longer pipettes and better colorimetry were introduced to decrease the analytical error in the hemoglobin determination. The method of Wong (7) was used for all iron determinations. Total blood phosphorus was determined by the modification of the Denigó method which we have recently described (8). We have measured the total blood phosphorus rather than merely the inorganic phosphate of the plasma since we felt any real increase of any of the various phosphorus compounds in the blood would be reflected in the total. If the total varied, later fractionation studies could be used to detect the class of phosphorus compounds responsible for the shift. We realized, however, that changes in the distribution might occur without changing the total.

In table 1 we have summarized the data of our second experiment. Although the refinement of our hemoglobin method has not altered our mean for the herd upon pasture, which represents the remnant of the herd upon which our first experiment was made, the values show a much smaller probable error.

Table 1 shows that in our herd there is no influence of either the summer sunshine or of pasture upon the hemoglobin, the iron or the total phosphorus of the blood of milking cows. After eighteen months upon rations that provide varying protein intakes there is no reflection in the blood constituents we have studied. This indicates that the minimum protein intake was adequate for the maintenance of the blood constituents that have been measured. We offer no explanation for the contrasts between our results and those of Kronacher other than the possibility of differences in herd management during the winter months. Our data show the very small differences that are found in the mean values for the hemoglobin, iron or total phosphorus values of the blood if an adequate number of animals is used in an experiment.

TABLE 1

Iron, phosphorus and hemoglobin in the blood of lactating cows before and after turning to pasture or dry lot in summer

	PASTURE	DRY LOT	HIGH PROTEIN	LOW PROTEIN	MEDIUM PROTEIN
Number of cows	35	39	13	13	13
Total P in May (mgm. per 100 cc.)	19 5±0 12	18 7±0 16	18 8±0 32	18 4±0 26	19 0±0 24
Total P in July (mgm. per 100 cc)	19 7±0 16	18 8±0 15	18 5±0 22	18 7±0 29	19 3±0 25
Fe in May (mgm. per 100 cc.)	39 5±0 39	38 7±0 48	39 2±0 89	38 9±0 92	38 9±0 64
Fe in July (mgm. per 100 cc.)	37 6±0 34	39 2±0 49	38 7±0 62	39 4±0 75	39 3±0 12
Hb in May (grams per 100 cc.)	11 0±0 06	11 7±0 14	11 7±0 19	11 7±0 29	11 9±0 19
Hb in July (grams per 100 cc.)	10 8±0 09	11 2±0 14	11 0±0 21	10 9±0 19	11 6±0 18

SUMMARY

By means of over a thousand determinations we have shown that the blood of the normal cow contains 10.9 ± 0.86 grams of hemoglobin per 100 cc. of blood. The blood of mature bulls is significantly higher in hemoglobin than that of cows. It contains 12.8 ± 0.8 grams per 100 cc. There is no relation between the hemoglobin of the cow and the following variables: breed, milk production, fat production, and the prolongation of the lactation period. There is no change in the hemoglobin of the blood that can be related to the period of gestation or lactation. No differences were found in total phosphorus, iron and hemoglobin in the blood of seventy-four cows at the close of the winter months and after a two-months' period of exposure to pasture or sunshine. No influence of an eighteen-months' feeding period with three levels of protein upon the hemoglobin, iron or total phosphorus of the blood of lactating cows could be found.

REFERENCES

(1) KRONACHER, C., BÖTTGER, TH., OGRIZEK, A., AND SCHAPER, W. 1927 Bluttrockensubstanz, Individuum und Rasse. Beitrage zur Frage der Verwendbarkeit der Bluttrockensubstanz als Kennmal der Individual- und Rassen Konstitution. Zeit. f. Tierzüchtung, viii, 183–227.

(2) KRONACHER, C., BÖTTGER, TH., AND SCHAPER, W. 1930 Korperbau Blutwerte, Konstitution and Leistung. Untersuchungen an ostfriesischen Kuhen. Zeit. f. Tierzüchtung, xvii, 177–244.

(3) COHEN, B., AND SMITH, A. H. 1919 The colorimetric determination of hemoglobin. Jour. Biol. Chem., xxxix, 489–496.

(4) ANDERSON, ARTHUR K., GAYLEY, HOWARD E., AND PRATT, AVERY D. 1930 Studies on the chemical composition of bovine blood. Jour. Dairy Sci., xiii, 336–48.

(5) SCHULZ, FR. N., AND KRÜGER, F. V. 1925 Das Blut der Wirbeltiere. Winterstein's Handbuch der Vergleichenden Physiologie. Jena, Bd. 1, p. 1128.

(6) VAN GELDER, R. H. 1928 Studies of the blood of cows in Switzerland and the Netherlands. Tijdschr. Diergeneesk, lv, 699–703.

(7) WONG, S. Y. 1928 Colorimetric determination of iron and hemoglobin in blood. Jour. Biol. Chem., lxxvii, 409.

(8) McCAY, C. M. 1931 Phosphorus distribution, sugar and hemoglobin in the blood of fish, eels and turtles. Jour. Biol. Chem., xc, 497–505.

THE CONTRIBUTION OF THE DAM IN INHERITANCE OF MILK AND BUTTERFAT*

LYNN COPELAND

Register of Merit Department, American Jersey Cattle Club, New York City

Raising the level of the average production of dairy cattle in America has in the past been accomplished primarily through improved feeding methods, better management, and through the elimination of low producing animals. Obviously, the degree to which the average production can be increased by these methods is limited. The results of Register of Merit and Herd Improvement Association testing during the past five years indicate that little further progress is being made by this means. To continue to raise this level of production it seems apparent that it is to inheritance and scientific breeding methods that cattle breeders and dairymen must look.

Modern breeding methods depend primarily on the selection and continued use over a period of time of prepotent herd sires. Genetic research has, during recent years, centered on methods of locating and choosing such sires. The herd sire has been given prominence by investigators since his offspring are far more numerous than those of any one cow, and he contributes one-half of the inheritance given to them. In much of the recent literature on the subject of inheritance of milk and butterfat production, the influence of the dam is almost ignored. Certainly the literature emphasizing the contribution of the dam from a genetic viewpoint is meagre compared to the amount that has been written relative to the sire. This is due not only to the greater number of progeny of the sire, but also because it is more readily possible to determine a bull's transmitting ability by the performance of his daughters.

Yet, judging from progress now being made it will be many years before any appreciable percentage of dairy calves are sired by proved bulls. In any event a great number of unproven young

---

* Received for publication February 11, 1931.

bulls must always continue to be used. Any proven bull was once an untried youngster and some breeder had to take a chance in proving him. It is a tragedy to the industry that so many of these good young prospective herd improvers are failures when proven. It is a challenge to constructive breeders and students of breeding to uncover additional facts and supply more information to dairymen that will materially assist them in selecting untried bull calves, calves that will in the majority of cases prove out successful.

A study of the influence or contribution of the dam involves a number of questions. The first of these is, just what is the importance of a production record of a cow? Has it any value from a breeding standpoint other than only indicating the transmitting ability of the cow's sire? When selecting an unproven sire, it is still the almost universal practice to give a great deal of consideration to the production record of the dam. Bull calves from dams with records over 1,000 pounds of butterfat are invariably in demand. This is because of the hope that the damwill transmit a measure of her own high producing ability to her son and through him to her granddaughters. Does this theory work out in actual breeding practice or is the percentage of failures so great as to render it of doubtful value? McDowell's and Parker's tabulations (1) show that as the production of the dams advances, the production of the daughters gradually climbs, but more slowly. This would indicate that there is at least a slight degree of relationship between the record of the cow and the records of her progeny. If it is true that the production record of a cow furnishes a fairly reliable measure of her transmitting ability, can it be assumed from this that there is a lack of dominance and that duplicate genes have a cumulative effect in inheritance of milk and butterfat production.

The theory has also been advanced that some of the hereditary factors governing milk and butterfat production are sex-linked and that possibly the dam should be given more consideration in the selection of a future herd sire. These and a host of other questions are involved in attempting to formulate any opinions on the ultimate value of a cow's record in the serious business of constructive cattle breeding. In view of the vital importance of the subject of increasing production it is the object of this study to present further data on the contribution of the dam and to determine if possible what conclusions might be drawn as to the relationship between her own producing ability and the producing ability of her daughters and granddaughters.

METHODS AND DATA

The materials used in this investigation were the records in the Register of Merit Department of the American Jersey Cattle Club. Since the establishment of test work nearly thirty years ago, over forty thousand long-time production records have been completed by Jersey Cows. The data on each of these records are still available in the files of the Register of Merit Department. In checking through these files it was found that to June 1, 1930, there were 694 cows that had production records themselves and also had at least one tested daughter and one Register of Merit son. (A son to be in the Register of Merit must have at least three tested daughters from different dams.) The highest records of each of these cows were converted to a mature 365 day basis. In doing this the records were first computed to a 365 day basis using the factor of 1.15 previously worked out in the Register of Merit Department (2). The records were then figured to a mature basis through the use of the age factors published by Fohrman (3). Likewise, the records of the daughters were converted to a uniform age basis. When the cow had two or more tested daughters, the records of the daughters were of course averaged. Similarly, the highest records of the sons' daughters were computed to a mature 365 day basis and then averaged.

When this preliminary work was completed, the records of the dams were compared with those of their daughters and with the records of their sons' daughters. The relationship or correlation between the dams' records and the sons' daughters' production will give an indication of the value of a production record in indicating or measuring the transmitting ability of the dam. The results of these comparisons are shown in tables 1 and 2 and

TABLE 1

Frequency table comparing records of dams and daughters

FAT PRODUCTION DIVISIONS OF DAMS	NUMBER OF COMPARISONS	AVERAGE YIELD OF DAMS	AVERAGE YIELD OF DAUGHTERS	DISTRIBUTION OF DAUGHTERS (POUNDS OF FAT)										
				Over 900	800–900	750–800	700–750	650–700	600–650	550–600	500–550	450–500	400–450	Below 400
pounds		*pounds*	*pounds*											
Over 900	26	959.86	678.00	1	4	1	3	6	4	2	4	1	0	0
800–900	54	844.63	683.15	2	6	6	9	11	6	8	4	2	0	0
750–800	39	772.81	600.02	0	1	1	5	5	7	6	7	6	1	0
700–750	55	721.85	616.24	0	3	1	2	13	15	10	4	4	2	1
650–700	77	671.63	596.23	1	7	3	4	10	8	7	19	12	5	1
600–650	77	625.44	583.35	1	1	5	4	15	6	14	9	10	8	4
550–600	69	578.66	558.34	1	0	6	3	3	7	11	14	12	9	3
500–550	93	525.46	544.25	0	1	1	3	11	10	15	20	15	11	6
450–500	84	479.10	516.14	0	0	1	2	5	6	10	21	19	13	7
400–450	73	431.86	532.98	0	1	1	3	6	7	7	18	14	12	4
Below 400	47	379.63	502.00	0	0	1	1	3	3	4	7	13	9	6
Totals...	694	603.30	572.97	6	24	27	39	88	79	94	127	108	70	32
Percentage distribution...				0.9	3.5	3.9	5.6	12.7	11.4	13.5	18.3	15.6	10.1	4.6

TABLE 2

Frequency table comparing records of dams and records of sons' daughters

FAT PRODUCTION DIVISIONS OF DAMS	NUMBER OF COMPARISONS	AVERAGE YIELD OF DAMS	AVERAGE YIELD OF SONS' DAUGHTERS	DISTRIBUTION OF SONS' DAUGHTERS (POUNDS OF FAT)										
				Over 900	800–900	750–800	700–750	650–700	600–650	550–600	500–550	450–500	400–450	Below 400
pounds		*pounds*	*pounds*											
Over 900	26	959.86	615.56	0	0	1	1	5	11	5	2	1	0	0
800–900	54	844.63	588.18	0	2	2	4	5	10	13	12	6	0	0
750–800	39	772.81	578.95	0	0	1	2	3	6	16	6	5	0	0
700–750	55	721.85	573.91	0	0	2	3	7	3	17	13	8	2	0
650–700	77	671.63	549.46	0	1	0	0	4	14	15	23	16	4	0
600–650	77	625.44	535.98	0	0	0	0	4	11	19	19	16	8	0
550–600	69	578.66	577.58	0	0	3	5	3	11	18	16	11	2	0
500–550	93	525.46	548.72	0	0	1	3	3	15	19	28	14	8	2
450–500	84	479.10	524.45	0	0	0	3	1	9	12	24	27	5	3
400–450	73	431.86	514.85	0	0	0	0	0	4	14	23	25	5	2
Below 400	47	379.63	488.62	0	0	0	0	0	4	2	9	20	8	4
Totals .	694	603.30	547.94	0	3	10	21	35	98	150	175	149	42	11
Percentage distribution.					0.4	1.4	3.0	5.0	14.1	21.6	25.2	21.5	6.1	1.6

figure 1. These tables also show the distribution of the records of the daughters and the records of the sons' daughters.

Examination of these tables and the accompanying figure shows that as the dams' records increase the production records of the daughters also increase and likewise the yield of the sons' daughters show some increase. However, the increase is much less relatively than that of the dams. The average production of all the mature (6 to 10 years) 365 day Register of Merit records that have ever been completed is 547.52 pounds of fat. This is shown by the horizontal line in figure 1. It should be noted that the average production of all of the sons' daughters is in almost exact agreement with the breed average. Comparing the records of the dams and daughters with the breed average it was found that for every pound the dams varied from the breed average the daughters varied about 0.32 of a pound, that is, when the dams were 100 pounds above the breed average, the daughters were approxi-

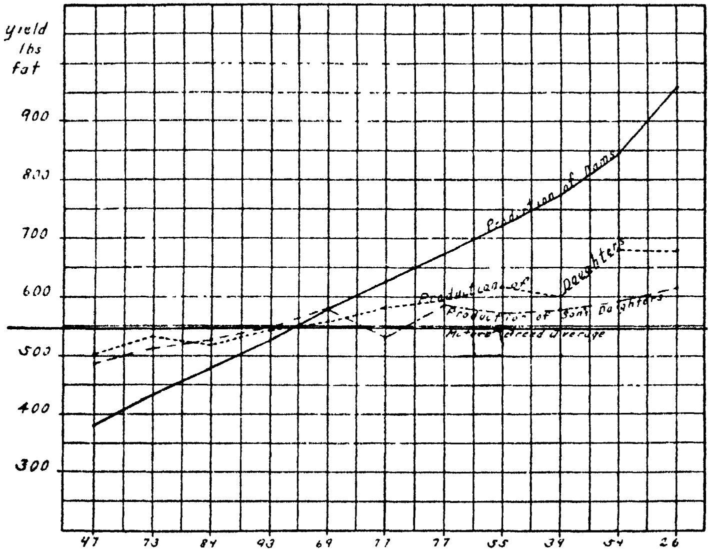


FIG. 1. COMPARISON OF RECORDS OF DAMS, DAMS' DAUGHTERS AND DAMS' SONS' DAUGHTERS

mately 32 pounds above the average. On a percentage basis the daughters showed 32 per cent as much variation from the breed average as did their dams. The frequency distribution of the records of the daughters and of the sons' daughters is shown much more clearly in figure 2.

It will be noted from the shape of the curve in the frequency polygon that the minimum entrance requirements for the Register of Merit exclude a significant number of the daughters and sons' daughters. This undoubtedly effects the direction of the daughters' curve and the sons' daughters' curve in the graph where the dams are below 550 pounds of fat and of course effects the coefficients of correlation between the production of the dams and the yield of their daughters and sons' daughters. If all the daughters and sons' daughters that failed to qualify were shown, the degree of correlation would be higher. Table 3 shows these constants for dams and daughters and dams and sons' daughters.

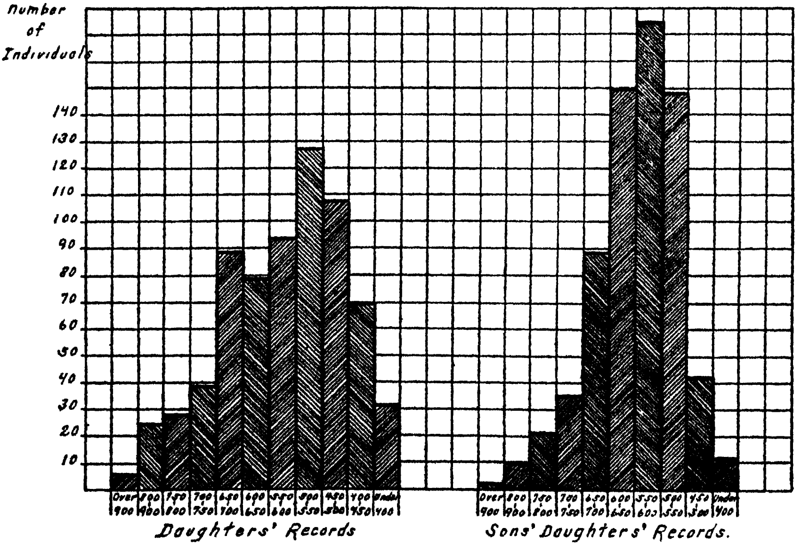


FIG. 2. FREQUENCY POLYGON SHOWING PRODUCTION DIVISIONS OF DAMS' DAUGHTERS AND DAMS' SONS' DAUGHTERS' RECORDS

From table 3 it will be seen that the coefficients of correlation between the dams and daughters and dams and sons' daughters while not extremely high are nevertheless very significant. Gowen (4) working with Holsteins reported a slightly higher correlation, (0.497 ±0.021) between dams and daughters and less correlation, (0.307 ±0.047) between maternal grandams and granddaughters. There are several reasons why the correlation between the dams and daughters should vary from that of the dams and sons' daughters. In fact, it is surprising that the variation is not greater than it is. One important reason is that the daughters of these cows were in the majority of cases tested in the same herds in which their dams were tested, while the daughters of the sons are more likely to be tested in different herds and under different conditions. The daughters of the sons have only about 25 per cent of their inheritance that would come through the dams while 50 per cent of the daughters' inheritance would come through the dams. Then too, these dams were probably rarely mated with sires possessing the same level of inheritance as they themselves had, consequently, even though the dams were homozygous, for their level of production the offspring are not likely to secure from their sires an inheritance for the same level of production as they do from their dams. In the case of the granddaughters this chance for an inheritance for a different level of production is introduced twice, not considering the possibility of the sons' dams being heterozygous, but considering only the chance of inheritance of different levels of production being introduced through the sons' sire and the sons' daughters' dams.

TABLE 3

Physical constants for dams and daughters and dams and sons' daughters

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	CORRELATION BETWEEN DAMS AND DAUGHTERS	CORRELATION BETWEEN DAMS AND SONS' DAUGHTERS
Dams .	603.30±3.89	151.88±2.75	2.52±0.046		
Daughters..	572.97±3.11	121.29±2.19	2.12±0.038	0.4044±0.0278	
Sons' daughters	547.94±2.21	75.50±1.56	1.38±0.029		0.3415±0.026

There is also a tendency for the better dams to be mated with better sires and the poorer dams with poorer sires.

While the correlation between the dams' records and the records of the daughters is undoubtedly somewhat higher than would be obtained were it possible to hold the influence of the sires fairly constant, on the other hand the elimination of low producing daughters through failure to meet the minimum entrance requirements tends to lower the degree of correlation that was found.

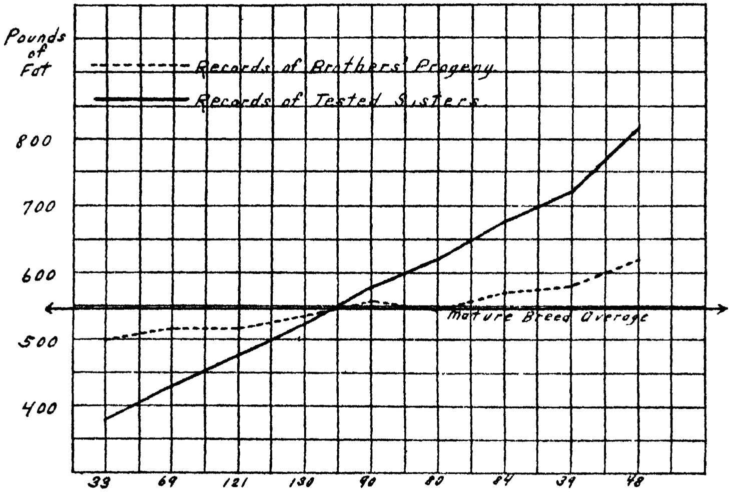


FIG. 3. RECORDS OF SISTERS COMPARED WITH RECORDS OF THEIR BROTHERS' DAUGHTERS

The data do indicate that the dams had to rank close to 600 pounds of butterfat in order to raise the production of their daughters above the mature breed average of 547 pounds while they had to average better than 700 pounds of butterfat in order for their sons' daughters to exceed the average mature production of the breed. It is evident that a cow's record is nearly twice as reliable a measure of the production of her own daughters as it is of her sons' daughters.

It was thought possible that a better index of the dam's ability to transmit production to her granddaughters through her sons would be the average production of all of her tested daughters. In other words, is it worth-while in selecting an unproven sire to give consideration to the records of his maternal sisters? In view of this a comparison was made between the records of the dams' daughters and the dams' sons' daughters. While it was not determined for the entire number, cursory examination revealed that in approximately 50 per cent of the cases the tested sisters were by the same sire as the proven son, and in the other 50 per cent the sisters were by different sires. No attempt was made to segregate the half-sisters from the full-sisters. Figure 3 shows the results of this comparison between the records of the tested sisters and the records of their brothers' progeny. The coefficient of correlation is shown in table 4.

TABLE 4

Physical constants for sisters' records and records of brothers' progeny

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	CORRELATION BETWEEN SISTERS AND BROTHERS PROGENY
Sisters' records	572 97±3 11	121 29±2 19	2 12±0 038	0 3679±0 025
Records of brothers' progeny	548 03±2 22	75 77±1 57	1 38±0 029	

It is surprising that the correlation between the records of the dams' daughters and the records of their sons' daughters is but little higher than that between the dams' own records and the records of their sons' daughters. For explanation, however, it may be added that in 131 instances the son had three or more tested sisters, in 196 cases there were two tested sisters, and in the remaining 367 instances there was only one tested sister. Davidson (5) concludes that it is necessary for a bull to have at least six unselected tested daughters in order to approximate his transmitting ability, more is very desirable. In view of this, it was not expected that the amount of correlation shown in table 4 would be very high. To date less than 300 Jersey cows are dams of four or more tested daughters and only 95 have five or more tested daughters. Nearly all of these dams are dead and hence an attempt to measure the inheritance or transmitting ability of a cow through a progeny performance test holds little possibilities.

Yet another indication of a cow's transmitting ability may lie in the production of her own tested sisters. To study the reliability of this index the records of the dams' paternal sisters were all converted to a mature 365 day basis. These records were then added to the dam's own record and the average determined. A better explanation might be that the average production of the dams' sires' daughters was computed. In 152 instances the dam either had no tested sisters or only one tested sister. Only the remaining 542 cases where the dam had at least two tested sisters were used in this analysis.

TABLE 5

Physical constants for dams' sisters, daughters and sons' daughters

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION	CORRELATION BETWEEN DAMS' SISTERS AND DAMS' SONS' DAUGHTERS	CORRELATION BETWEEN DAMS' SISTERS AND DAMS' DAUGHTERS
Dams' sisters..	531.34±2.02	60 89±1 43	1.15±0.027		
Dams' daughters.........	576.86±3.92	118.39±2 77	2 05±0.048		0 4046±0.028
Dams' sons' daughters. .	549.30±2.66	80.36±1.88	1 46±0.034	0.3440±0.029	

At first the individual dam's record was compared with the average record of all of her sire's daughters, (her own record included). The correlation coefficient was found to be 0.561 ± 0.022. The mean fat production of the dams was 603.30 ±3.89 while that of her sire's daughters was 531.34 ±2.02 showing a difference of 71.96.

The average production records of the dams' sires' daughters were then compared both with the dams' daughters and also with the dams' sons' daughters. The results of this comparison are given in figure 4 and table 5.

In examining table 5, it is worth-while and significant to note how closely the coefficient of correlation between the dams' sisters (their own records included) and the dams' daughters compares with the correlation between the dams' own record alone and the records of her daughters. The same close degree of correlation also exists between the records of the dams' sisters (their own records included) and the dams' sons' daughters and the correlation between the dams' records alone and the records of the sons' daughters. (Refer to tables 3 and 5.) This is not in exact agreement with Turner's findings (6) in which he reports that the relationship between the dam's sire's daughters and the dam's son's daughters is much better than the relationship between the dam's own record and the production of her son's daughters.

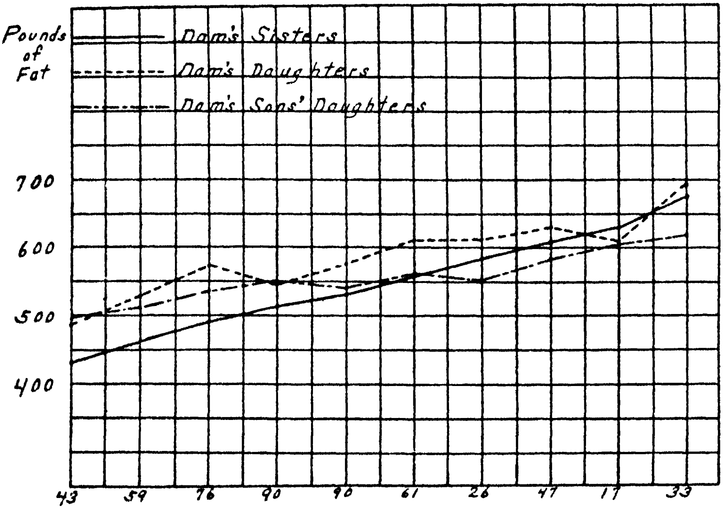


Fig. 4. Comparison of Records of Dams' Sisters with Dams' Daughters and Dams' Sons' Daughters

In reviewing the foregoing tabulations it will be noted that in predicting the producing ability of a bull's daughters the bull's dam's record, the records of a few maternal sisters, and the records of his dam's sisters all rank about equal. This led to the thought that possibly by considering all three of these factors a more reliable indication of a bull's probable transmitting ability might be obtained. To develop this possibility the average production of the dam's daughters and the average yield of the dam's

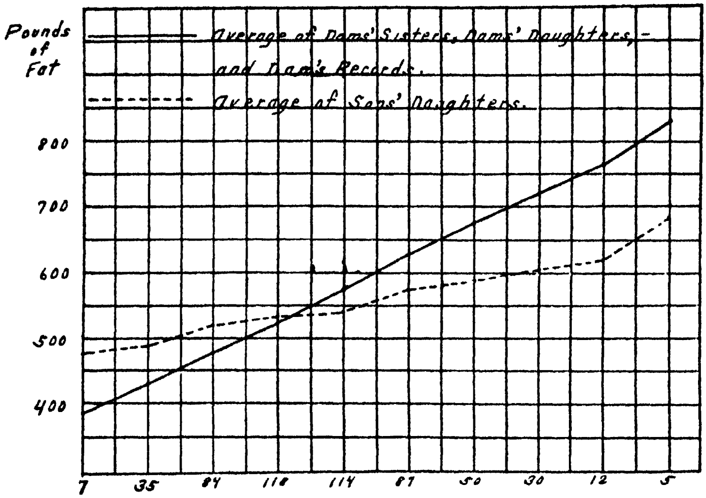


FIG. 5. COMPARISON OF AN AVERAGE OF DAMS' RECORDS, DAMS' SISTERS AND DAMS' DAUGHTERS' RECORDS WITH RECORDS OF DAMS' SONS' DAUGHTERS

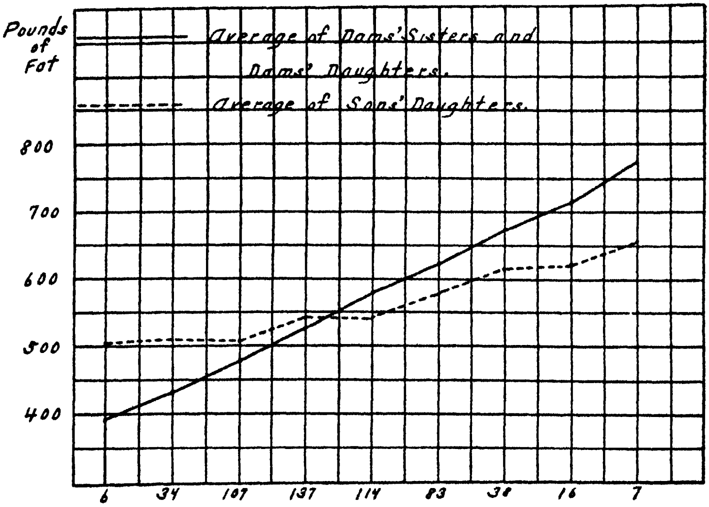


FIG. 6. COMPARISON OF AN AVERAGE OF DAMS' SISTERS' AND DAMS' DAUGHTERS' RECORDS WITH RECORDS OF DAMS' SONS' DAUGHTERS

sire's daughters were averaged with the dam's own record. The average figure thus obtained was compared with the average records of the sons' daughters. A total of 542 comparisons were made, the cases in which the dams had no tested sisters being excluded. The coefficient of correlation between the average figures obtained by the foregoing method and the sons' daughters' records was found to be 0.412 ±0.027. This degree of correlation is considerably higher than any of the previous comparisons made with the bulls tested progeny. Figure 5, also shows this graphically. This correlation emphasizes that consideration of not only the dam's record but also the average yield of her sisters and the production of her daughters is very advisable in selecting an untried young bull. Of course, it has been shown (7) that equal or more attention should also be given to the sire but a discussion of the sire's contribution was not a part of this study. The results of this comparison suggested and prompted a similar comparison but omitting the dam's own record. That is, the average production of the dam's sire's daughters was added to the average production of the dam's daughters and the result divided by two. The average figure obtained was then compared with the sons' daughters' records, 542 comparisons being made. In this case the coefficient of correlation was 0.409 ±0.028. This is nearly as good correlation as was secured when the dam's record was included. Figure 6 indicates the relationship.

SUMMARY

The significance of these results to practical breeding may be summarized as follows:

1. It is evident that there is some correlation between a cow's producing ability and the producing ability of her daughters. Selection of females from high record dams should result in a herd that will produce in excess of the breed average. In general the daughters show about 32 per cent as much variation from the breed average as do the dams.

2. There is a slight correlation between the dam's record and the records of her sons' daughters. However, it appears that very little progress can be hoped for if sires are selected solely

on the basis of their dam's records. It is obvious that a production record considered by itself is a poor index for the germinal composition of the dam for the characters for production. The record of the dam is nearly twice as reliable a measure of the production of her own daughters as it is of her sons' daughters.

3. The records of only a very few tested maternal sisters does not alone furnish a reliable guide for predicting the transmitting ability of an untried bull.

4. There is some correlation between the average production of the dam's half-sisters by the same sire and the average production of the dam's sons' daughters. However, selecting a bull with but this in mind is not recommended from the results of this investigation.

5. In choosing any untried young bull consideration should not be limited to the sire alone but the maternal ancestry is worthy of careful study. *Too much information cannot possibly be obtained.* The results of the last phase of this investigation indicate that when an average is made of the dam's record, the dam's sisters' records and the dam's daughters' records the average figure obtained in this manner provides a more accurate and reliable index as to a bull's probable transmitting ability than does any one of these factors considered by itself. If the dam has no record the average of her maternal sisters' records and of her daughters' records gives just about as dependable results.

6. In purchasing an untried young bull it is now generally recommended, in the light of present knowledge, that first of all he be sired by a proven bull, a bull whose daughters are uniformly high producers and superior to their dams. After this fact has been verified attention should then be given not only to the dam's record but also to the records of her sisters and the records of her daughters. Selection of a bull by this method seems to provide the most possibilities for success.

REFERENCES

(1) McDowell, J. C., and Parker, J. B.: Better cows from better sires. U. S. D. A. Dept. Circular 368, 1926.

(2) Copeland, Lynn: Comparison of 305 and 365 day records. Jersey Bulletin, September 29, 1926.

(3) Fohrman, M. H.: Factors for adjusting milk and butterfat records of Register of Merit Jersey cows to a uniform age basis. Jour. Dairy Sci., September, 1926, ix, no. 5.

(4) Gowen, John W.: Milk Secretion. Williams and Wilkins, Publishers, 1924.

(5) Davidson, F. A.: Measuring the breeding value of dairy sires by the records of their first few advanced registry daughters. Ill. Agr. Exp. Station Bulletin no. 270, 1925.

(6) and (7) Turner, C. W : A comparison of Guernsey sires. Mo. Agr. Exp. Station Research Bulletin 79, 1925.

UNIFORM RULES FOR THE HERD TEST*

The American Dairy Science Association at its summer meeting ast June at Ames, Iowa, considered and adopted the recommendations of its Breeds Relation Committee regarding uniform herd test rules in connection with the new Herd Improvement Tests. The Uniform Herd Test Rules were developed by the special committee of the Official Testing Section after several special meetings had been held with representatives of the different breed associations. The purpose of the Uniform Herd Test Rules is stated as follows:

1. To standardize and simplify the methods of obtaining and supervising herd tests for economical production of dairy cows of all breeds.
2. To encourage mass testing of dairy herds under herd conditions.
3. To make the records more reliable and more easily comprehended and interpreted.
4. To make possible recognition and greater use of worthy dairy herd sires through production records of all daughters.

The following are the suggested rules:

Entering herds on test. Any owner of registered cattle of a dairy breed may make application at any time to the National Breed Association concerned to enter his herd on test, using forms furnished by the breed association. All cows of milking age, both registered and grade, must be included in the herd test.

Identification of cows on test. All cows on test must be identified by tattoo number or by color sketches on broken-colored animals. The day, month, and year of birth must be reported on all registered animals, and on grades if possible; if not, approximate dates for grades will be accepted.

Conduct of test. The herd test may be started at the first of any calendar month. Twelve consecutive periods of twenty-four hours each, approximately one month apart, without preliminary

---

* Received for publication February 27, 1931.

milking, or six consecutive periods of twenty-four hours each, approximately two months apart with preliminary milking, shall be required during the testing year, retests and surprise tests not included. The tester shall indicate on his report which system of testing was used. Parts of two consecutive lactation periods of any cow made within the testing year may be included in the yearly average. Credits for milk may begin with the fourth day after calving, but no butterfat test shall be made before the seventh day. The day on which the calf is dropped is counted as the first day. Single samples are to be taken, each sample to represent each milking of each cow during the twenty-four hours of the test period and each tested for butterfat content.

Number of milkings. Cows may be milked not more than three times daily. Not more than two cows shall be milked at one time and the cows must be so placed that the supervisor can observe the milking of the cows continuously. In no case shall more than 48 milkings be supervised in one day—24 cows if milked twice daily, or 16 cows if milked three times daily. The tester's report must show the number of times each cow is milked during the testing period.

Retests. Retests may be required when any herd averages 40 pounds of butterfat per cow in thirty days, or when after the second month after calving any cow produces in excess of the following:

	Lbs fat in one day
Junior 2-year old	1 7
Senior 2-year old	1 9
Junior 3-year old	2 0
Senior 3-year old	2 1
Junior 4-year old	2 2
Senior 4-year old	2 3
Mature cows	2 5

Whenever a herd, or individual cow, produces more than these minimum requirements, the tester must notify the state superintendent of testing immediately after the completion of the test. If one or more cows in the herd require a retest the state superintendent in charge may require that the entire herd or any portion thereof be retested.

Surprise tests. One or more surprise tests with preliminary dry milking may be made during the year. Surprise tests shall be made by a different supervisor than the one conducting the previous month's regular test on the herd. The expense of one surprise test, if made, and all retests shall be borne by the owner. Any additional surprise tests will be at the expense of the breed association.

Feed cost and value of product. The owner shall coöperate with the tester by furnishing information regarding value of product, rations used, and local values or prices paid for feed. The tester shall ascertain the weight and record the amounts of all feed consumed by the individual cows, as well as the number of days on pasture. Monthly computations of feed, costs, and income shall be made by the tester and copied by him into a herd record book which shall remain in the possession of the farmer. A duplicate copy of this monthly record shall be furnished on demand to the breed association concerned.

Computing yearly and monthly herd averages. In calculating herd averages the production of all registered first calf heifers that have been in milk ten or more months of the herd test year, and all registered cows that have ever freshened and that have been owned in the herd ten months or more of the herd test year, shall be included in the herd average.

In reporting herd averages, a supplementary list shall be furnished showing all heifers and new cows in the herd less than ten months, together with their production.

Dishonest or fraudulent practice. If the state superintendent of testing is satisfied that fraudulent or dishonest practices have been used in the making of herd test records of registered cows, he shall report same to the breed association which may reject or cancel such records.

Matters not covered by rules. Details of supervision which these rules do not specifically cover shall be administered by the state superintendent of official testing.

Revision of rules. Any revision of rules shall be made by a joint committee of the breed associations and American Dairy Science Association. The chairman of the Breeds Relation Committee of the Official Testing Section of the American Dairy Science Association shall be ex-officio chairman.

E. L. ANTHONY,
Chairman, Official Testing Section,
J. B. FITCH,
Chairman, Breed's Relation Committee.

THE CHURN AS A SOURCE OF MOLDS IN BUTTER*

H. MACY, W. B. COMBS AND H. B. MORRISON, JR.

Division of Dairy Husbandry, University of Minnesota, St. Paul, Minnesota

Unquestionably, the typical wooden churn found in our creameries today is the most highly contaminated and the most difficult to sterilize of any dairy equipment. Little has been done to determine the actual extent of infection, particularly by making studies of a dissected or dismantled churn to locate the outstanding areas of contamination or foci of infection, especially concerning molds.

Gregory (1), Haglund and his co-workers (2), Macy and Combs (6), James (4), Paraschtschuk (7), Hood and White (3), Lund (5), and Stiritz (8), have indicted the churn as a prolific source of infection of butter.

It was quite evident from a study of the literature that there was an opportunity to make a more intimate study of typical churns, in order to obtain a better idea as to the presence or absence of molds in various places in the churn itself. The following studies were undertaken with this objective in mind.

Two churns were available for study. Both of them had been in regular use in commercial creameries for a period of years and were obtained at the time they were discarded. Churn A was a Giant Disbrow that had been used for about ten years in a creamery where, previous to its removal, there had been experienced considerable difficulty with moldy butter and excessively high mold and yeast counts. Churn B was a Master Dual that had been in daily use at the Minnesota State Experimental Creamery for a period of seven years, during which no particular difficulty with molds or yeasts had been encountered. Churn A was obtained for study in July, 1928, and Churn B in July, 1930.

---

* Published with the approval of the Director as Paper No. 984, Journal Series, Minnesota Agricultural Experiment Station. Received for publication March 4, 1931.

METHODS

Churn A. After its arrival at University Farm, it was found that the inner surface showed numerous areas of visible mold of many types. In order to remove as much as possible, the inside of the churn was washed thoroughly and treated with boiling water and steam for several hours. The mechanical parts were then removed and the churn again washed inside and out with boiling water. Following this, the barrel was cross sectioned at each end by means of an ordinary hand saw. This made it possible to gain access to various parts of the churn with little difficulty. The various parts of the churn were then examined, dissected in a variety of ways, and samples taken from every conceivable place. Scrapings were made from all metal parts or exposed places such as bolt heads, sight glasses, ends of rollers or shafts, stuffing boxes, surface bruises, crevices behind stationary parts, gates, etc. The staves were then carefully removed serially, at both ends and from the middle section. Similarly the parts of each drum were separated. By means of a chisel, which was sterilized in an open flame before each sample was taken, blocks of wood were removed from various places in solid wood areas and adjacent stave surfaces. In the latter case, samples of wood were removed to include each quarter inch from the inner churn surface toward the joint of the staves, which was approximately one inch from the surface. Altogether, 230 representative samples were taken. The samples were placed in sterile 4-ounce, aluminum-capped glass jars.

Churn B. Immediately after the last churning was made, this churn was washed and scalded by the routine method employed by the creamery. Samples from various accessible places were taken immediately. The churn was then removed and the various mechanical parts dismantled as far as possible. The following morning samples were taken from various parts of the churn including scrapings from all surfaces, metal parts, roller ends, etc. The rollers and shelves were taken out promptly and appropriate samples taken. Portions of wood from shelves, drum, staves, etc., were then withdrawn with the greatest possible care by means of a flamed chisel. Especial effort was made to obtain sections of wood at the surfaces of adjacent, representative staves completely around the barrel. Altogether, 73 samples were taken. These were placed in sterile test tubes and stored in ice until ready for examination.

In both studies, a sufficient quantity of sterile water was added to each jar or tube so that the sample was completely immersed. They were allowed to stand in this way overnight. Platings of 1 cc. of the fluid were made and poured with acidulated whey agar in the usual manner. Incubation was for five days at room temperature. No attempt was made to get quantitative results. The mere presence of mold was to be the criterion.

PRESENTATION OF DATA

Churn A. Of the total of two hundred thirty samples taken, one hundred ninety-two carried molds. The wood at the joints between staves at various depths, up to 1 inch from the inner surface of the churn; wood from cracks in barrel, doors, workers, and shelves, whether at the surface or ½ inch below the surface; wood from the sides or ends of shelves; wooden shelf braces; wooden rolls or workers; scrapings from bolts; metal brackets, inner sides of castings over the ends of workers and in the surrounding or underlying wood, etc., were found to carry molds of one sort or another. The joints in the drums were not as heavily contaminated, this probably being due to the fact that the individual pieces are held more firmly together. The fact that the mycelium of the mold apparently penetrates as far as an inch into the joints between staves is important since those areas present a vast total of possible infected foci.

A representative collection of cultures was obtained from the plates prepared from the samples taken from Churn A. These cultures were identified generically as far as possible and were found to represent eighteen different genera, many of which have been reported as having been isolated from butter. The following genera were found: Aspergillus (9 cultures); Alternaria, Monilia, and Penicillium (7 cultures each); Mucor and Sporotrichum (6 each); Hormodendrum (5); Acremoniella, Botrytis, Phoma, and Spicaria (3 each); Glioclardium, Oospora, and

Spondylocladium (2 each); and one each of Cephalosporium, Chaetomium, Sordaria, and Tilachlidium. Fourteen other cultures produced no fruiting bodies and were not identified.

Churn B. Altogether, 73 samples were taken. Only 22 specimens showed any signs of being contaminated with molds and only 5 of these produced more than two mold colonies on plating. These latter included scrapings from débris behind one of the doors, wood from right upper cradle arm, scrapings from the outer side of the left sight glass, bolt in metal band of right drum, and housing of left end of upper roll shaft. The types of molds present were Mucor, Penicillium, Aspergillus, Alternaria, and Hromodendrum. Although one might expect to find considerable numbers of *Oospora lactis,* it was strikingly scarce in Churn A and absent in samples from Churn B.

While this report does not concern itself with yeasts or bacteria in the churn, it might be mentioned that the former were quite consistently present in the great majority of cases and the latter always, sometimes in large numbers.

DISCUSSION

The results presented here demonstrate that a churn that is in bad condition may harbor considerable numbers and varieties of molds that may prove detrimental to the market quality of butter made in it, if such molds are able to develop. On the contrary, if the churn is kept in good condition by daily use and thorough treatment, it need not constitute a very serious menace. Nevertheless, new churns are not always free from mold, as shown by an examination of a churn recently purchased by the University. The types of molds found in the churns were those which often are responsible for the typical moldy butter where discolored areas mar the appearance and market value of the product. The comparative absence of *Oospora lactis,* which is the typical form encountered on plates prepared for routine mold counts leads one to believe that this mold is indicative of the presence of remnants of cream, butter, or buttermilk in the churn, which in turn means that the churn has not been thoroughly cleaned or has not been in daily use.

The presence of mold in joints between staves at depths of $\frac{3}{4}$ to 1 inch from the inner surface of the churn is quite significant since it demonstrates that the mycelium must continue its foothold in such remote places and when conditions are favorable, extend hyphae toward the surface for fructification to take place. Consequently, when a churn is allowed to remain idle and especially to dry out so that the staves shrink and the creavices open, the opportunity is presented for this situation to arise. Under ordinary conditions, the bands placed upon the barrel of the churn hold the staves tightly together, but long-continued use of the churn may lead to considerable loosening and subsequent establishment of infected foci.

The crevices between the boards in the end of the churn do not appear to be as highly contaminated as other parts of the churn. This may be due to the fact that the boards are held more tightly in place so that less space is left for the introduction of infection.

The excessive strain which is placed upon the churn during the butter working process undoubtedly results in the dislodgment of contaminated material from various areas involved with the moving parts, such as rollers, and particularly about the packing and bolts. It has been common experience to find that the contamination of butter is greater during the working period than it had been while the rollers were idle.

The reason that species of molds other than *Oospora lactis* seldom appear in considerable numbers on plates made for mold and yeast counts is undoubtedly due to the high dilution on the cream and its fractions, butter-buttermilk. The very nature of the scattered discolored areas indicative of moldiness of butter is another demonstration of this fact.

The evidence presented in this paper makes it clear that a churn may be an important source of mold in butter and should receive careful attention by the buttermaker in his methods of sanitation to reduce the contamination from this particular piece of equipment.

SUMMARY

1. Two churns from commercial creameries were studied to determine the presence of mold.

2. Molds of several types were found in various places in both churns, especially in Churn A, which had been known to be giving trouble.
3. The genera of molds present in the churns are listed.
4. It is shown that the churn may be an important source of mold in butter.

REFERENCES

(1) Gregory, H W.: The churn as a factor in the contamination of butter. N Y Prod Rev. and Amer Creamery, i, 18, 22, (1920).

(2) Haglund, E., Barthel, C , and Waller, E : Karnans skotsel och det framstallda smorets kvalitet och hållbarhet. Meddel. Centralanst Forsoksv. Jordbruksomradet (Sweden), No 297, (1926) Original paper examined Translation by C L. Brooke

(3) Hood, E G , and White, A H : The cause and prevention of mould in Canadian pasteurized butter. Canada Dept Agr Bul 48, n s , (1925)

(4) James, Norman: A study of churn sanitation Sci Agr , ix, 649–55, (1929)

(5) Lund, T H : Yeasts and mold in butter. N Y. Prod Rev. and Amer. Creamery, xl, 1184-92, (1920)

(6) Macy, H , and Combs, W. B : Field studies of the sources of mold in butter. Minn. Agr Ex Sta Bul. 235, (1927)

(7) Paraschtschuk, S : Report of the work of the Northwestern Territory Experimental Laboratory in dairying in Leningrad for the years 1926-27. (Translated title) Rept N. W. Exptl. Lab in Dairying, 1926-27, Leningrad, pp 26–33; 34–44. Original paper examined. Translation by P. L Dutko

(8) Stiritz, B. A : Yeast and mold counts and their relation to pasteurization of cream for buttermaking purposes Jour. Dairy Sci., v, 362–71, (1922).

PRELIMINARY STUDIES OF CHURN SANITATION*

H. B. MORRISON JR., H. MACY AND W. B. COMBS

Division of Dairy Husbandry, University of Minnesota, St. Paul, Minnesota

It has been demonstrated clearly that the churn is an important source of contamination of butter. Once the various parts of the churn become infected, it is an extremely difficult task to eliminate the contamination or even to control the situation satisfactorily. The problem of churn sanitation has been before the industry for many years, and no universally acceptable method for controlling this difficulty has been suggested. The studies here reported were undertaken to serve as a basis for further investigations with the objective of finding the most desirable and most satisfactory method of treating churns to remove or control the infected areas.

Previous studies of churn sanitation have shown that no particular method has proven to be most satisfactory. Gregory (2), Lund (6) (7), Shutt (8), Hood and White (4), Haglund, Barthel, and Waller (3), James (5) and workers at the Indiana Station (1), have studied the problem in various ways and have demonstrated that the sanitation of the churn is exceedingly important and likewise difficult to accomplish.

METHODS

In the experiments here reported, preliminary studies were made of various methods of treatment. A No. 2, Dairy Disbrow churn, with a capacity of 90 gallons of cream, which had been idle for some time, was selected for use. There was visible mold growth at various places on the inner surfaces. In addition, broth cultures of *Alternaria humicola*, *Aspergillus niger*, *Aspergillus*

---

* Received for publication March 4, 1931.

The data upon which this paper is based were taken from a thesis presented by H. B. Morrison, Jr., in partial fulfillment of the requirements for the degree of M. S., University of Minnesota. Published with the approval of the Director as Paper 985, Journal Series, Minnesota Agricultural Experiment Station.

flavus, *Hormodendrum clasdosporioides*, *Oospora lactis*, *Penicillium biforme*, *Penicillium expansum*, *Mucor sylvaticus*, and *Rhizopus nigricans* were introduced into the churn and allowed to grow several days before treatments began. It was desired to contaminate the churn excessively and to permit the molds to spread as extensively as possible so that extreme conditions would prevail. Thereafter, treatments with hot water, steam, hypochlorites, and chloramines were carried out. These methods will be discussed more in detail under separate headings later. In general, before each treatment, the churn was rinsed with three gallons of tap water. Following this rinse, the hot water or chemical solution was placed in the churn. When steam was used, the hose was placed in the churn, the door laid across the hose, and high pressure steam allowed to pass directly into the churn. Following the various treatments, a gallon of autoclaved skimmilk was used to rinse the churn. This material provided a good medium for the growth of any surviving organisms and in the instances where chemicals were used, it provided for the absorption of excess chlorine, thus eliminating any residual effect of the chlorine on the plates prepared from the samples.

Representative samples for plating were taken of the rinse water from the churn before the treatment and of the skimmilk rinse after the churn had been treated. Check samples were also taken regularly of the water, chemical solutions, and autoclaved skimmilk. In a few cases the water and chemical solutions were not absolutely sterile, but not sufficiently contaminated to vitiate results.

The samples were plated directly using whey agar as the culture medium. The medium for the mold plates was acidulated in the usual manner with tartaric acid. The plates were incubated for five days at room temperature.

The results were not recorded quantitatively, but qualitatively, to give a relative idea of the amount of infection. The symbols used in recording the results were as follows: (−) no growth; (+) a few colonies; (+ +) many colonies; and (+ + +) very many colonies.

PRESENTATION OF DATA—PRELIMINARY HEAT PENETRATION STUDIES

Inasmuch as hot water has been commonly used for churn treatment, it was considered desirable to make a preliminary investigation of the penetration of heat into wood from a churn.

In the first study, adjacent holes were bored in a representative stave (2 inches thick) of a standard churn, one clear through the stave so that the thermometer bulb was immersed in the water inside the churn, a second to within ½ inch from the surface in contact with the water, and a third to within 1 inch from that surface. The churn was filled with water at 80°C. and kept at that temperature for two hours. At the end of this period, the temperature recorded at a depth of ½ inch was 63°C., while that at 1 inch was only 53°C.

TABLE 1

Rate of heat penetration of churn wood

TIME OF EXPOSURE	TEMPERATURE OF WATER	TEMPERATURE OF WOOD AT VARIOUS DEPTHS		
		¼ inch	¾ inch	1¼ inch
minutes	°C.	°C.	°C	°C
Start	71.2	25.9	25.9	25.6
10	96.8	47.4	30.8	26.4
20	96.8	62.2	41.7	31.3
30	97.6	69.8	50.6	37.9
40	97.2	74.8	57.6	44.7
50	97.5	77.4	62.9	50.3
60	97.5	80.1	66.8	54.5
70	97.3	81.6	69.8	57.8
80	97.1	82.6	71.9	60.3
90	97.4	83.6	73.7	62.3
100	97.3	84.3	75.0	63.8
110	97.5	84.4	75.8	64.8
120	97.7	85.2	76.4	65.7

These results led to a further study in which a portion of a stave from the same churn and about 6 inches long and 2 inches thick was imbedded in plaster of paris in such a way that only one surface (the typical inner surface, as in the churn) was exposed to the water in the water bath. Holes were bored in the stave parallel to the exposed face and at distances of $\frac{1}{2}$, $\frac{7}{8}$, and $1\frac{3}{8}$ inch from the surface in contact with the water. A few drops of mercury were placed in each hole to insure good contact with the bulbs of thermometers inserted in these holes. Hot water was placed in the water bath and brought to the boiling point as quickly as possible by means of a Bunsen burner placed beneath it. The temperature of the water and wood at the various depths was recorded every five minutes during a period of two hours. Three trials were made. These checked very closely and the results presented in table 1 are averages of the three trials.

As one might expect, there was a lag phase, at the beginning of the exposure, which was more pronounced as the distance from the point of contact increased. In all cases at least 75 per cent of the rise in temperature took place during the first hour. After that, the rise was comparatively slow, and finally the increase was very slight during succeeding intervals. As noted, it required twenty minutes to bring the wood at a depth of $\frac{1}{2}$ inch to the temperature of pasteurization, fifty minutes at $\frac{7}{8}$ inch, and an hour and a half at a depth of $1\frac{3}{8}$ inch, which represents the point at which the staves are jointed. As shown in a previous paper by the authors, since the crevices between churn staves are sometimes infected with mold as far from the surface as these joints, it is obvious that it would be necessary to expose the churn to excessively long treatments with hot water in order to be sure that the molds at such a depth were destroyed.

CHURN TREATMENT STUDIES

a. Treatments with hot water

In the majority of creameries, hot water is, usually, directly available or easily prepared by heating water with steam, and has been most generally used for churn treatment. In the trials reported, the test churn was subjected to several rinsings such as it might receive in an ordinary creamery. Thereafter the treatments were applied.

Trial 1. In this, as in subsequent trials, the churn was revolved during the exposure, for various periods of time. As will be noted in table 2, the water rinse of the churn previous to exposure to the hot water indicated that the churn was highly contaminated with molds and bacteria. The rinse, obtained after treatment by using sterile skimmilk while the churn was being run for several revolutions, showed that the treatment with water at a temperature ranging from 78.5° to 88°C. was not sufficient to destroy the microörganisms present.

Trials 2, 3, 4, and 5. The amount of water and the periods of exposure were increased in these cases. Otherwise, the same methods used in Trial 1 were repeated. The results obtained were identical, as indicated by the data in table 2.

TABLE 2

Churn treated with hot water

	TRIAL 1	TRIAL 2	TRIAL 3	TRIAL 4	TRIAL 5	TRIAL 6
Amount of water in churn (gallons)...	15	30	30	30	30	30
Fullness of churn..	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$
Temperature of water at start (°C.)...	88	88	85	87	88	96
Temperature of water at finish (°C.)...	78.5	80.7	77.1	77 8	73 9	87.0
Period of exposure (minutes)	5	10	20	30	60	30
Churn rinse before treatment (water):						
Molds.........	+++	+++	+++	+++	+++	+++
Bacteria..............	+++	+++	+++	+++	+++	+++
Churn rinse after treatment (skim milk):						
Molds.................	+++	+++	+++	+++	+++	–
Bacteria	+++	+++	+++	+++	+++	+++

Trial 6. In this case, the temperature of the water at the beginning of the period of exposure was brought to 96°C. by introducing steam into the water and bringing the temperature to the desired point before beginning the thirty minute exposure. The effectiveness of this higher temperature is evidenced by the successful results reported in table 2.

Trials 7, 8, and 9. In order to place the method of treatment on a more practical basis, it was decided to continue the hot water treatment on successive days rather than to permit an interval to elapse between exposures during which the molds might develop fruiting bodies. Table 3 gives the data obtained in these trials. The churn in this case was filled with water which was kept practically at the boiling point during the treatment by means of the introduction of steam at frequent intervals. At the beginning of the trial, the churn was visibly contaminated with mold. The observation on the third day indicated that no conidia were present. The bacteria were in this case somewhat reduced as well. This trial indicated clearly the advantage of regular, daily treatment of the churn.

In order to determine whether or not the improvement was permanent, the churn was allowed to stand idle for four days following these successive treatments, It was rinsed each day with enough cold water to submerge the rollers. After revolving the churn for several minutes, samples of the rinse water were taken, and the remainder was discarded. It was found that on each succeeding day the evidences of reëstablishment of mold and bacterial growth were more noticeable. Apparently, the mycelia of the molds had not been destroyed by the hot water. The fruiting bodies and free conidia on the surface of the wood were the structures which suffered from the exposure.

TABLE 3

Churn treated with hot water on successive days

	TRIAL 7	TRIAL 8	TRIAL 9
Amount of water in churn (gallons)	90	90	90
Fullness of churn	Full	Full	Full
Temperature of water (°C)	97.7	97.2	97.7
Period of exposure (minutes)	180	180	180
Churn rinse before treatment (water):			
Molds	+++	*	–
Bacteria	+++	*	+++
Churn rinse after treatment (skim milk).			
Molds	–	–	–
Bacteria	++	++	++

* Samples lost.

b. Treatments with steam

Trial 10. After the churn was given the usual preliminary rinsing with cold water, the steam hose was introduced through the door opening. The door was then closed over it. The steam was allowed to flow into the churn slowly for a period of three hours. The results given in table 4 clearly show that the treatment was quite successful against the molds. Again, the effect was not permanent, as rinsings taken from the churn four days afterward contained large numbers of molds.

Trials 11, 12, and 13. The same method of steam treatment was applied on three successive days with results as indicated in table 5. As before, the advantage of regular, daily treatment is brought out clearly in this experience. The control of surface growth is very definitely the outcome of an uninterrupted routine of churn treatment.

TABLE 4

Churn treated with steam

	TRIAL 10
Temperature of steam in churn (°C.)	97 7
Period of exposure (minutes)	180
Churn rinse before treatment (water):	
Molds	+++
Bacteria	+++
Churn rinse after treatment (skim milk):	
Molds	—
Bacteria	+++

TABLE 5

Churn treated with steam on successive days

	TRIAL 11	TRIAL 12	TRIAL 13
Temperature of steam in churn (°C.)	97 7	97 7	97 7
Period of exposure (minutes)	180	180	180
Churn rinse before treatment (water):			
Molds	+	—	—
Bacteria	+++	+++	+++
Churn rinse after treatment (skim milk):			
Molds	—	—	—
Bacteria	+++	+++	++

Following the successive treatments with steam, the churn was again allowed to remain idle and untreated for several days. Observations made daily thereafter showed that the molds quite soon began normal fructification and the inner churn surface in a short time was spotted with visible areas of mold.

c. Treatments with sodium hypochlorite solutions

Since the use of various chlorine-containing preparations has been quite widely advocated, particularly by commercial concerns, experiments with representative types of these compounds were undertaken.

Trial 14. In the first trial, a solution of sodium hypochlorite containing 54 p.p.m. of available chlorine was used. During the period of exposure, the available chlorine dropped to 35 p.p.m.

TABLE 6

Churn treated with sodium hypochlorite solutions

	TRIAL 14	TRIAL 15	TRIAL 16	TRIAL 17
Amount of solution (gallons)	30	90	30	90
Fullness of churn	⅓	Full	⅓	Full
Available chlorine in solution at start (p.p.m.)	54	35	200	265
Temperature of solution (°C.)	15.6	10.0	15.6	10
Period of exposure (hours)	½	18	½	18
Churn rinse before treatment (water):				
Molds	++	+++	+++	+++
Bacteria	++	+++	+++	+++
Churn rinse after treatment (skim milk):				
Molds	++	+++	+++	+++
Bacteria	++	+++	+++	+++

The results given in table 6 show that the solution had no noticeable effect upon the molds or bacteria.

Trial 15. Using the same methods, but with a larger amount of solution, somewhat lower in available chlorine, and over a longer period of time, the results obtained were negative as shown in table 6.

Trials 16 and 17. Inasmuch as solutions with the concentration of available chlorine commonly recommended did not give successful results, much stronger solutions were used. The results were negative.

d. Treatments with solutions of alkaline, crystalline hypochlorite

Trials 18, 19, 20, and 21. A type of hypochlorite in crystalline form in combination with tri-sodium phosphate was used in solutions of various strengths. Concentrations considered as ordinary and high were used during long and short exposures. The results given in table 7 indicate definitely that the solutions were

TABLE 7

Churn treated with solutions of alkaline, crystalline hypochlorite

	TRIAL 18	TRIAL 19	TRIAL 20	TRIAL 21
Amount of solution (gallons)	60	90	60	90
Fullness of churn	$\frac{2}{3}$	Full	$\frac{2}{3}$	Full
Available chlorine in solution at start (p.p.m.)	62	53	283	274
Temperature of solution (°C)	10	13 3	15 6	12 2
Period of exposure (hours)	2	18	2	18
Churn rinse before treatment (water):				
Molds	+++	+++	+++	+++
Bacteria	+++	+++	+++	+++
Churn rinse after treatment (skim milk):				
Molds	+++	+++	+++	++
Bacteria	+++	+++	+++	+++

TABLE 8

Churn treated with chloramine-T solutions

	TRIAL 22	TRIAL 23	TRIAL 24	TRIAL 25
Amount of solution (gallons)	30	50	90	90
Fullness of churn	$\frac{1}{3}$	$\frac{5}{9}$	Full	Full
Available chlorine in solution at start (p.p.m.)	53	46	300+	350
Temperature of solution (°C.)	51 6	51 6	48 8	48 8
Period of exposure (hours)	2	18	2	18
Churn rinse before treatment (water):				
Molds	+++	+++	+++	+++
Bacteria	++	+++	+++	+++
Churn rinse after treatment (skim milk):				
Molds	+++	++	+++	+++
Bacteria	+++	+++	+++	+++

not effective in destroying the surface molds in the churn. As in the case of ordinary sodium hypochlorite solutions, there was a noticeable decrease in available chlorine during the time the solutions were in the churn.

e. Treatments with chloramine-T solutions

Trials 22, 23, 24, and 25. A more stable chlorine-bearing compound, a chloramine-T was employed, in these trials in solutions of various concentrations and for different periods of time. None of these trials gave satisfactory results as will be noted in table 8, although there was a very slight improvement in the conditions in the churn in Trial 23. The strength of the solution diminished during the longer periods of exposures. Altogether, the chloramine solutions used were ineffective.

DISCUSSION

It has been established beyond all doubt that churns do become infected with various microörganisms. The problem then resolves itself into a search for the most effective methods for reducing this infection to a minimum.

The results presented in the preceding pages indicate very definitely that of the methods used, sufficient exposures to water at sufficiently high temperatures and freely flowing steam were the most satisfactory.

In the preliminary studies on the heat penetration of wood, it was evident that it required considerable time for the temperature of the wood to be raised to a point sufficiently high to be effective in destroying even vegetative forms of microörganisms. Since the infected areas may be at some distance from the inner surface of the churn, it is obvious that this factor must be taken into account if all infection is to be overcome. Under practical creamery conditions, it might be considered too costly and too laborious a task to use sufficiently large quantities of hot water. Such extreme exposures might have an undesirable effect upon the churn itself. A possible modification of prolonged treatment is suggested as the result of the trials. Where moderate quantities of water, sufficient to cover the rolls, are used at high temperatures and for reasonable periods of time, comparatively satisfactory results are obtained if such treatment is repeated daily without interruption. Under these conditions, the surface of the wood at least is kept in a good sanitary condition, and fructification from concealed mycelia of molds especially is inhibited. With the churn in daily use and with the wood thoroughly swollen so that the normal expansion tends to keep the crevices between staves, etc., more nearly closed, the daily treatment with hot water gives reasonably satisfactory results. This daily treatment cannot be over-emphasized.

In the case of steam, satisfactory results were obtained, especially when the treatment was applied without fail each day. With steam, however, the effects upon the churn, especially the blistering of the paint, may be sufficient to discourage its use.

The use of chlorine-bearing solutions gave negative results consistently. Even with high concentrations of available chlorine, the effect upon the microflora of the churn was scarcely noticeable. In the experimental churn used in these tests, conditions under which chlorine compounds had to work were not nearly as bad as they usually are in regularly used commercial churns, where the presence of a film of butter fat makes it more difficult for the solution to reach the microörganisms. Based upon the results obtained in these studies, solutions of sodium hypochlorites, crystalline alkaline hypochlorites, or chloramines could not be recommended. In the case of the alkaline washing solutions in general, there is an undesirable effect upon the wood which discourages their use. With these alkaline solutions there appears to be a softening of the wood surface which leads to difficulty.

The fact that treated churns when allowed to stand idle for several days become a serious source of infection for cream must not be overlooked. Regardless of the apparent effectiveness of any method of treatment as measured by the examination of skimmilk used as a rinse immediately after treatment, the organisms were not completely eliminated, as studies made several days later showed the churn to be very much infected. Treatment merely improved the conditions but did not remove the cause.

The molds were more readily destroyed than the bacteria. In fact, in no instance were the bacteria eliminated. Apparently, heat- and chlorine-resistant forms established themselves in the churn and resisted all efforts to overcome them. Hot water and steam were most effective against them.

In general, the results obtained in these preliminary studies with a highly infected churn demonstrate that the most satisfactory method of treatment used was water at a high temperature for a sufficient period of time and repeated daily without interruption. Further investigations are to be made using other methods and expanding the work to include trials on churns in daily use in commercial creameries.

SUMMARY

1. Various methods for treating a churn to rid it from micro-organisms were investigated.
2. When the churn was partially filled with water at 96°C. and revolved for one-half hour, the surface molds were destroyed.
3. The hot water treatment was most effective when carried out daily.
4. Failure to treat the churn daily resulted in the reappearance of mold on the inner surface of the churn.
5. The use of steam for sufficient periods of time was effective against the molds but caused the paint on the churn to blister badly.
6. Solutions of sodium hypochlorite, alkaline crystalline hypochlorite, and chloramine-T were ineffective.
7. None of the treatments was especially effective against bacteria.

REFERENCES

(1) Anon.: Germ content of the creamery churn. Indiana Agr. Expt. Sta. Rept., 1923, pp. 24, 25, (1923).

(2) Gregory, H. W.: The churn as a factor in the contamination of butter. N. Y. Prod. Rev. and Amer. Creamery, i, 18, 22, (1920).

(3) Haglund, E., Barthel, C., and Waller, E.: Karnans skötsel och det framställda smorets kvalitet och hållbarhet. Meddel. Centralanst. Försöksv. Jordbruksområdet (Sweden), No. 297, (1926). Original paper examined. Translation by C. L. Brooke.

(4) Hood, E. G., and White, A. H.: The cause and prevention of mould in Canadian pasteurized butter. Canada Dept. Agr. Bul. 48, n s., (1925).

(5) James, Norman: A study of churn sanitation. Sci. Agr., ix, 649–55, (1929).

(6) Lund, T. H.: Yeasts in pasteurized cream butter. Creamery and Milk Plant Monthly, x, No. 1, 30, (1921).

(7) Lund, T. H.: Buttermakers interest in micro-organisms. N. Y. Produce Rev. and Amer. Creamery, liii, 693–8, (1922).

(8) Shutt, D. B.: Studies on the sterilization of churns. Michigan Agr. Expt. Sta. Rpt., 1924–25, pp. 182–191, (1926).

THE SOLUBILITY OF METALS IN MILK

I. THE SOLUBILITY OF COPPER UNDER VARIOUS CONDITIONS*

H. T. GEBHARDT AND H. H. SOMMER

The harmful effect of dissolved copper in dairy products on their flavor and keeping quality is now quite generally recognized. Experimental results and practical experience have demonstrated that dissolved copper is capable of producing a "cardboard" or "cappy" flavor in fresh milk and cream, and that dissolved copper is an important factor in the development of tallowy and, in some cases, fishy flavors in such products as butter, condensed milk, frozen cream, and whole milk powder. The solubility of copper in dairy products under various conditions is therefore of direct practical importance.

Equipment constructed of copper, copper alloys and tinned copper is in extensive use in the dairy industry. This is true because of installations that were made before the deleterious effect of copper was recognized as it is at present, and because of new installations in which such metals are used on account of relative cheapness, mechanical advantages in the working of the metals and high heat conductivity. Such use of copper equipment may not be disastrous in all cases because the rate of copper solution is variable. Accurate information is therefore desirable concerning the factors that affect the rate of copper solution so that the conditions may be recognized under which copper equipment may or may not be used.

DISCUSSION OF PREVIOUS WORK

The solubility of copper in milk has been studied by various investigators (1, 5, 6, 7, 8, ,9, 11, 12). These studies have revealed many data and interesting facts on copper solubility in

---

* The work was supported in part by a grant from the Special Research Fund of the University of Wisconsin.

Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

milk, but unfortunately the value of this information is greatly reduced (*a*) the data of the various investigators are not comparable, and (*b*) their experimental methods are more or less open to criticism.

The most comprehensive studies have been carried out by Rice and Miscall (12), and later by Quam et al. (11) and by Miscall et al. (7). However, the data on the amounts of copper dissolving in milk under certain conditions do not agree or are not comparable. Quam et al., reported the temperature of maximum solubility of copper in milk as 85°C. in 1928 (11), and as 90°C. in 1929 (9), while Miscall et al. (7) reported it as 66°C. in raw milk 60°C. in pasteurized milk. Quam et al. exposed the copper to the milk for thirty minutes, and Miscall et al., for one-hundred and twenty minutes. Quam attributed the lower temperature of maximum solubility as reported by Miscall et al., to chemical changes that occur in the milk during the longer exposure.

The method used by Rice and Miscall (12) and by Miscall et al. (7) for exposing the copper to milk was to place 14 twisted strips of copper, 1 by 5 inches, into 2 liters of milk in a 5-liter flask, for two hours without agitation. It is known that the relative velocity of the liquid to the metal is an important factor in metal corrosion; Frazer et al. (3) found that the rate of corrosion increases with the velocity and approaches constancy when a velocity of 200 feet per minute is attained. In view of this, the lack of mechanical agitation in the above investigations must be considered a serious fault. The only agitation was that resulting from convection currents, and as such must have varied with the temperature and other conditions. These considerations limit the value of the investigations cited above, and the data obtained are comparable only with data obtained by a similar procedure.

The method used by Quam et al. (11) for exposing the copper to milk, provided an alternating flow of the milk over the crimped metal test pieces. A higher relative velocity of liquid to metal might have been desirable; but the main criticism of this procedure is that the aeration of the exposed film of milk (*a*) affected the gaseous content so that the results cannot be considered comparable to submerged corrosion tests, and (*b*) provided opportunity for evaporation so that the temperature of the exposed film was undoubtedly lowered, especially in the tests conducted at higher temperatures. These criticisms are especially applicable in view of the corrosion factors as enumerated by Frazer et al. (3), which emphasize that in corrosion tests the important factors are the composition of the liquid including dissolved gases, the relative velocity of the liquid to the metal, and the temperature.

Rice and Miscall (12) have reported that previously boiled milk dissolves less copper than raw milk, while Miscall et al. (7) have reported that pasteurized milk dissolves more copper than raw milk. No explanation is offered by the above investigators for these phenomena, but the suggestion is made that these are probably related to chemical changes produced in the milk by the heating.

These phenomena are undoubtedly related to the reduced solubility of copper in milk at temperatures beyond that of maximum solubility. Quam (9), citing Speller (13), suggested that the break in the temperature-solubility curve of copper in milk is due to the decreased solubility of oxygen in milk at higher temperatures. This suggestéd explanation can hardly be adequate, because there is no such break in the solubility curve of oxygen in water as given by Speller (13).

PURPOSE OF THE PRESENT STUDY

The present experimental work was undertaken (*a*) to review the findings of previous investigators, (*b*) to accumulate additional data on the solubility of copper in milk under various conditions, (*c*) to seek the causes of the reduced solubility of copper in milk at temperatures above the point of maximum solubility, and the changed solubility in preheated milk, and (*d*) to gain a better theoretical understanding of the processes involved in the solution of copper in milk.

EXPERIMENTAL METHOD USED

a. Apparatus and methods for corrosion tests

The method for exposing the copper to the milk was designed to give staisfactory control of (*a*) the composition of the milk, (especially gaseous content), (*b*) the relative velocity of the test pieces to the milk, and (*c*) the temperature of the milk. This includes all the fundamental factors which are known to determine the usefulness of laboratory corrosion tests (Frazer et al. (3).

The metal test pieces were either thin sheets of copper 1 by 2 inches (these were used in all of the first experiments) of 25.8 $cm.^2$ surface, or else, samples of commercial sheet copper 1 by 3 inches, $\frac{1}{32}$ inch thick, of 39.66 $cm.^2$ total surface.

These test pieces were exposed to 500 cc. milk,[1] contained in 1 quart milk bottles *1*. (See diagram, *fig. 1.*) A stirring device passing through rubber stopper *2* entered the neck of the bottle. The stirring device consisted of supporting arm *3*; bearing *4*, shaft *5*, chuck *6*, collars *7*, fly wheel *8*, and attachment *9*, of the flexible cable *10* to the shaft. A glass stirring rod *11*, was held firmly by chuck *6;* the glass rod *11* was provided with small side-arms of glass (upper arms *12*), and over the lower end of the rod fitted the glass tube *13* which was also provided with side-arms of glass (lower arms *14*). This lower tube was held in position by the upward pull of the rubber bands *15*, suspended from the hooks *16* on the chuck. The metal test pieces *17* were held in small grooves of the side arms between the upper and lower side arms by the tension of the rubber bands *15*. Cup *18* was provided to collect any condensate water flowing down from the upper metal parts of the bearing and the chuck. Through glass tubes *19*, gases of the desired nature could be passed through the milk. There was a hole in the side of the bottle to permit the entrance of the thermometer *20*, an electrode *21*, and a salt bridge *22* (saturated KCl agar) through the stopper *23*.

The closure at *2* was airtight and did not permit any exchange of gases because the bearing was 3 inches long, closely fitting, and was kept well oiled.

Four bottles of this type were prepared. All four stirring shafts were driven through flexible steel shafts by an electric motor. The speed of the motor was reduced, and the drive thus obtained was at the rate of 210 r.p.m. for all four shafts.

---

[1] In certain cases 530 cc. milk was used.

These bottles were placed either in separate water-baths or else, as in the later experiments, all were placed in the same water-bath. The water-baths were heated with gas, with the heating flame regulated by means of a toluol—mercury thermo-regulator, which kept the temperature constant within 1°. Deviations from this rule will be reported in connection with the presentation of the experimental results.

In the experiments where the thin copper sheets were used, these were fastened by pinching them between a 2 mm. glass rod and the stirring rod, which were held together by the tension of small rubber bands. In this manner practically the entire 25.8 $cm.^2$ of copper surface was exposed, only a narrow line between the two glass rods being covered. Although the number of revolutions was constant, the relative velocity was not uniform at various places over the copper surface; it was higher at the periferal portion, and lower towards the center. These conditions, however, were quite uniform as indicated by the uniformity of the results obtained.

These conditions were improved in the method of attachment used for the larger copper blades. These were held rigidly in a vertical position parallel to the stirring rod and about 1 inch from the center. This arrangement facilitated the exchange of the test pieces. Both sides of the copper blades were exposed at nearly the same velocity, and only a relatively small area of the metal was unexposed, viz. the small area at the edges in contact with the holding arms.

This arrangement provided as nearly ideal conditions of uniform velocity as can be expected. Absolute uniformity of velocity over the entire surface would necessitate test pieces to fit perfectly the stream lines in the liquid. It appeared practically impossible to avoid entirely any unexposed areas at the points of attachment. Evans (2) proposed a method for accomplishing this; but because of the chance of slight losses due to abrasion of the metal, and for various practical reasons, the method of Evans was not followed.

In the present arrangement all the controllable variables as described by Frazer et al. (3) have received consideration. A

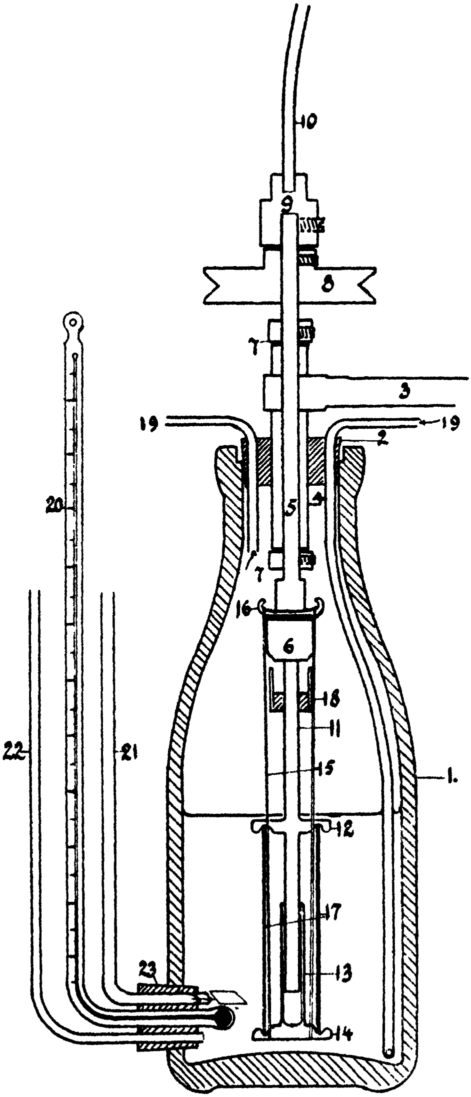


Fig. 1

definite and sufficiently large quantity of the corrosive liquid was provided. The temperature of the water bath and that of the milk was controlled. Further, this arrangement permitted the bubbling of gases of the desired nature through the milk, practically the same amount of gas passing through all four bottles when connected in series. It is recognized that a higher velocity (revolutions per minute) might have been chosen to good advantage and that some device might have been provided to prevent the continuous circular flow of the milk.

It is known that the condition of the metal surface influences metal solution. Therefore, an attempt was made to prepare the metal surfaces so as to furnish the same type of surface in every test. This was done for the 1 by 2 inch thin copper sheets by cleaning in dilute nitric acid, or later by short immersion in bichromate-sulphuric acid cleaning mixture, followed by rinsing in water. The copper sheets were then placed in fresh milk for five to ten minutes, until they were used for the test. The larger copper blades were treated in a different manner. The method chosen was intended to simulate the nature of well cleaned metal surfaces in dairy equipment. The test pieces were cleaned by scouring with "Bon Ami," just enough to remove any tarnish, but care was taken not to bring about a polishing effect.

b. Method for determining the amount of copper dissolved

The amount of copper dissolved was determined in two different ways. In the earlier experiments, when thin copper sheets were used, the milk was ashed and the copper in it was determined by analysis using the method as already published (4). These results were originally expressed in terms of milligrams of copper dissolved in 1 liter of milk after exposure to the 25.8 $cm.^2$ of copper surface for two hours. Later these results were expressed as milligrams of copper dissolved per square decimeter of copper surface per hour.

In the later experiments the losses were determined directly from the difference in weight of the test pieces before and after exposure. The metal test pieces after cleaning in the manner as described above were prepared for weighing by rinsing in alcohol and finally in ethyl ether. Before they were weighed, they were kept in a desiccator at room temperature for at least one hour. The weighed test pieces were then placed between the holders of the stirring device, and subjected to the test. After exposure they were quickly removed, and rinsed in cold tap water. Any adhering milk constituents were removed by means of a cotton swab. The blades were then rinsed in distilled water, blotted dry, rinsed in alcohol, and finally in ether. Before weighing they were again kept in a desiccator for at least one hour. The results were finally expressed in terms of milligrams loss per square decimeter, per hour.

EXPERIMENTAL RESULTS

1. *The effect of acidity on the solubility of copper in milk*

It is generally assumed that the acidity of the milk is an important factor in the solution of metals by milk. The study of the effect of hydrogen-ion concentration was therefore given first consideration. In the experiments that were conducted on the effect of acidity, this factor was varied and the temperature and other factors were kept constant. The experimental details and the results are presented in table 1. A study of the data leads to the following conclusions:

1. Small variations in acidity produced no perceptible effect on the solubility of copper in milk.
2. Higher acidities reduced the solubility of copper.
3. Neutralizing the milk to an alkaline reaction did not perceptibly influence the solubility of copper.
4. The effect of acidity is the same whether it is produced by the addition of pure lactic acid or by biological agencies.

The experiments were primarily intended for comparison within each series only; the samples within each series were taken from the same lot of milk. A comparison between the series, however, is possible and quite instructive. Samples *a* to *d*, which were skim milk, when compared with all the other samples, which were whole milk, indicate that copper is less soluble in skim milk than in whole milk. Other factors may have been involved such as differences in the gaseous content occasioned by differences in the age and previous handling of the milk. However, it should be noted here than all the milk samples were taken from the University herd milk in which the bacterial count was known to be well below 10,000 per cubic centimeter, and the samples were handled in such a manner as to exclude secondary effects due to bacterial growth.

TABLE 1

The effect of acidity on the solubility of copper in milk

SAMPLE	pH	TITRATABLE ACIDITY; LACTIC ACID	ACIDITY PRODUCED BY:	TEMPERATURE	COPPER CONTENT IN MGM /L	COPPER DISSOLVED IN MGM / DM ^{2}/HR *
		per cent		°*C.*		
a	6.54	0.17–0.16	Nothing added	20	0.69	0.55
b	6.54	0.17–0.16	Nothing added	20	0.62	0.49
c	5.72	0.33–0.32	Dilute lactic acid	20	0.69	0.55
d	5.72	0.33–0.32	Same as c	20	0.62	0.48
e	4.58		19.2 cc. N/1 lactic acid	19.5	1.14	0.97
f	5.00		15.0 cc. N/1 lactic acid	19.5	1.02	0.86
g	5.30		6.6 cc. N/1 lactic acid	19.5	1.55	1.37
h	6.30		Nothing added	19.5	1.43	1.32
i	6.56		Nothing added	20	1.28	1.06
j	3.28		Dilute lactic acid	20	0.60	0.41
k	3.28		Same as j	20	0.69	0.49
l	8.42		Dilute NaOH	20	1.23	1.01
m			Pasteurized milk, nothing added	20		0.90
n	4.03		Ripened 24 hours	20		0.82

Experimental details:

Surface of copper: 25.8 cm.^{2} for samples a–l; 39.7 cm.^{2} for samples m and n.

Time of exposure: Two hours for samples a–l; one hour for samples m and n.

Amount and kind of milk used: 500 cc. skimmilk for samples a–d; 500 cc. whole milk for samples e–l; about 500 cc. whole milk for samples m and n.

* Dissolved copper was calculated based on the determined copper content of the original milk in samples e–h, (0.136 mgm. per liter), and in samples i–l, (0.182 mgm. per liter), or calculated by assuming that the copper content of the original milk was 0.12 mgm. per liter for samples a–d, and 0.15 mgm. per liter for samples m and n.

2. The effect of dissolved gases on the solubility of copper in milk

It has been demonstrated by various investigators that the dissolved oxygen in the corrosive liquid plays an important rôle in metal solution. Thus it is apparent that the gaseous constituents of milk, which may be ignored in many dairy manufacturing problems, are likely to be of utmost importance here. With this in mind experiments were undertaken to determine the effect of evacuation, aeration, and of treatment with oxygen, and carbon dioxide, on the solubility of copper in milk. The results and experimental details are given in table 2. The following conclusions may be drawn from the results obtained:

TABLE 2

The influence of dissolved gases on the solubility of copper

SAMPLE	TREATMENT	TEMPERATURE	COPPER CONTENT OF MILK IN MGM./L	COPPER DISSOLVED IN MGM./DM.^{2}/HR.*
		°C		
a	None	Room	0.493	0.37
b	Vacuum, 4 hours	Room	0.440	0.31
c	Air	Room	0.834	0.66
d	None	Room	0.562	0.40
e	None	20.5	0.683	0.52
f	None	20.5	0.714	0.55
g	Carbon dioxide	26.0	0.317	0.16
h	Oxygen	26.0	1.645	1.45
i	Oxygen	23.0	1.580	1.38
j	Oxygen	62.8	12.240	11.70
k	Oxygen	62.8	(Direct gravimetric)	17.65

Experimental details:

Surface of copper: 25.8 cm.^{2}, except in sample k, where it was 39.7 cm.^{2}

Time of exposure: Two hours, except in sample k, where it was one hour.

Amount of milk used: 500 cc. skimmilk was used in samples a and b, fresh whole milk in all other samples.

* For calculating the dissolved copper, the original copper content of the milk was assumed to be 0.15 mgm. per liter for whole milk, and 0.12 mgm. for skimmilk.

1. Evacuation of the milk previous to exposure to copper slightly decreases the amount of copper dissolved.

2. The bubbling of air through the milk during the exposure to copper increased the amount of copper dissolved very slightly.
3. The bubbling of carbon dioxide through the milk during the exposure to copper very markedly decreased the amount of copper dissolved.
4. The bubbling of oxygen through the milk during exposure to copper appreciably increased the amount of copper dissolved. The effect was more pronounced at pasteurizing temperature than at room temperature.

3. The effect of other milk constituents on the solubility of copper in milk

No experimental study was made of the effect of other milk constituents on copper solubility, although they are undoubtedly involved in the corrosive action. However, the individual milk constituents, in all probability, do not exert their effects independently, and therefore any attempt to study the isolated constituents singly would lead to erroneous conclusions. If a study of the effect of individual milk constituents were to be undertaken, it would be necessary to vary the amount of one in the presence of constant amounts of all the other constituents. In view of the immense amount of work that would be required in preparing the samples and in studying this phase of the subject adequately, in view of the fact that the milk constituents in mixed milk are reasonably constant (certainly as far as corrosive effect may be concerned), and in view of the fact that the results could not lead to any practical control of the problem, no work of this nature was undertaken. Nevertheless it is of interest to speculate on the rôle of the various milk constituents in metal solution.

The effect of the organic constituents appears to be of an indirect nature. Lactose is a reducing sugar and as such might be expected to exert an effect on copper solution. However, its reducing effect is pronounced only in alkaline solutions, and therefore probably plays no important rôle in the solution of copper by milk.

The milk fat cannot take part directly in any ionic process such as metal solution, yet there are indications that whole milk has a greater solvent action on copper than skim milk (see table 1, compare samples *a* to *d* with *e* to *n*). More data are needed before a definite conclusion may be drawn concerning the effect of fat in milk on copper solution.

The protein substances in the milk are only slightly ionized, and would therefore appear to be indifferent in the process of metal solution. However, insoluble protein salts of the heavy metals may be formed, thereby promptly removing the dissolved metal from solution. As a result proteins are likely to affect metal solution indirectly.

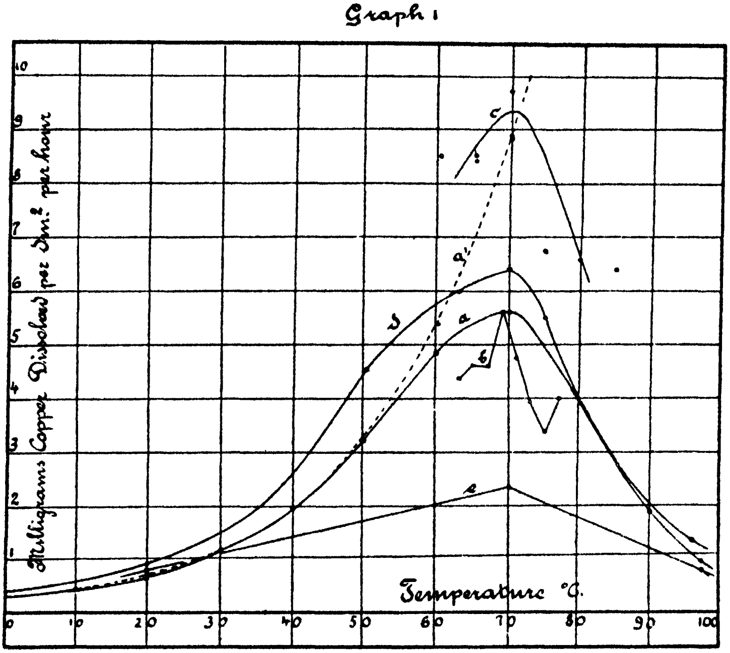
In this connection it is of interest to recall that the colloidal constituents of milk bear electric charges which bring about the migration of these particles under the influence of an external electromotive force. Metal salts may influence the electric charges sufficiently to alter the properties of the milk as a colloidal suspension or emulsion. These phenomena are probably related to the difference in the behavior of metal surfaces with respect to the baking on of the milk solids.

The ionic constituents of the milk, viz. the salts, appear to be most directly involved in the solution of metals in milk, because the salts comprise the anions which may combine with the metal, and determine the hydrogen-ion concentration. From later studies, however, it appears that the salts are not the most important factors in metal solution.

4. The effect of temperature on the solubility of copper in milk

The velocity of chemical reactions increases with the temperature. For this reason the rate of copper solution in milk is expected to increase with the temperature. Previous investigators have shown that this expected effect is realized only up to a certain temperature, beyond which there is actually a decrease in the amount of copper dissolved. However, there is a lack of agreement concerning the temperature of maximum solubility. Further, no explanation, founded on adequate experimental evidence, has been offered to account for the decreased solubility of copper at temperatures beyond that of maximum solubility. Experimental results on this subject are therefore of special interest both from a theoretical and practical standpoint.

The experimental results on the solubility of copper in milk at various temperatures during exposures of thirty, sixty, and one



The Effect of Temperature on Copper Solubility in Milk.

FIG. 2. *a*. Curve showing copper solubility in mgm./dm.^{2}/hr., calculated from gravimetric determinations of the dissolved copper in milk after two hours exposure to 25.8 cm.^{2} surface of copper at temperature intervals of 10°C.

a' Curve showing copper solubility in mgm./dm.^{2}/hr., calculated from the formula:

$$\text{Log } R = a + b \cdot t^\circ; \text{ Log R} = 1.414 + 0\ 02195\ t^\circ$$

where R = mgm. Cu/dm.^{2}/hr. *a* and *b*—calculated from the experimental data at 10° and at 40°C.

b. Same as *a*,—but at 2° temperature intervals from 63° to 77°C.

c. Same as *a*,—but thirty minutes exposure only.

d. Curve showing copper solubility in mgm./dm.^{2}/hr., calculated from the losses in weight of strips of plate copper of 39.7 cm.^{2} surface, exposed to milk for one hour at 20°, 60°, 62.8°, 70°, 75°, 85°, and 97°, average of 4 to 8 determinations.

e. Same as *d*,—but in acid milk at 20°, 70°, and 97°C.

hundred and twenty minutes, are presented in figure 2. A study of this figure and of the data on which it is based leads to the following conclusions:

1. Temperature is the most important of the factors that determine the amount of copper dissolving in milk. Up to a temperature of 50°C. the solubility increases at a logarithmic rate as expressed in the temperature-reaction velocity equation:

$$\log R = a + b.\ t^{\circ}$$

2. The temperature of maximum solubility is 70°C.; this holds true whether the time of exposure is thirty minutes, one hour, or two hours. This finding is contradictory to the contention of Quam, that upon exposures longer than thirty minutes, chemical changes occur in the milk which change the temperature of maximum solubility.

3. The rate of copper solution becomes less as the time of exposure is increased.

4. The decrease in the solubility of copper in milk at higher acidity, as observed at room temperature (see table 1), is much more marked at higher temperatures.

It should be noted that the above conclusion refers to the solubility of copper and not copper oxide, as on a tarnished surface. The good results obtained by the use of hot, sour whey for cleaning tarnished copper equipment are not to be explained by the specific action of sour whey on copper, but rather on the copper oxide which apparently is dissolved quite readily by sour whey. However, sweet milk also dissolves films of copper oxide quite readily.

5. The effect of the previous heat treatment of milk on copper solubility

Rice and Miscall (12) have reported not only that less copper dissolved in milk at boiling temperature than at room temperature, but also that milk, after it had been boiled and cooled, dissolved less copper than raw milk. Raw milk dissolved 16.25 mgm. copper per liter at room temperature, while milk previously boiled and cooled at room temperature dissolved 5.35 mgm. copper per liter, and saturation with air in the latter case increased the amount only to 5.45 mgm. This enormous difference in the solubility of copper apparently was related to some change in the composition of the milk. A study of these phenomena promised to elucidate some of the fundamental factors that influence the solubility of copper in milk.

The findings of Rice and Miscall were partially confirmed by the results of the experiment presented in table 3, but the differences found between the solubilities in raw and previously boiled milk were slight at room temperature, and even at boiling temperature, they were not so great as reported by these investigators. Their results are probably to be attributed to the lack of agitation in their experimental procedure. An experiment of a similar nature (table 3, samples *e* to *h*) showed that previous pasteurization did not decrease copper solubility, but rather increased it slightly.

TABLE 3

The solubility of copper in preheated milk

SAMPLE	HEAT TREATMENT OF MILK	TEMPERATURE	COPPER CONTENT OF MILK IN MGM /L	COPPER DISSOLVED IN MGM /DM ^{2}/HR ‡
		°C.		
a	Raw milk	Room	0 737	0 60
b	Raw milk	Boiling	2 535	2 34
c	Previously boiled*	Room	0 615	0 48
d	Previously boiled	Boiling	1 561	1 40
e	Raw milk	Room	0 515	0 38
f	Raw milk	62 8	3 945	3 68
g	Previously pasteurized†	Room	0 845	0 68
h	Previously pasteurized	62 8	4 005	3 83

Experimental details:

Surface of copper: 25.8 cm.^{2}

Time of exposure: Two hours

Amount of milk used: 500 cc. (skimmilk).

* The milk was heated to about 97°C. in a boiling water bath for about twenty minutes, then cooled.

† The milk was previously pasteurized by heating to 62.8°C. for thirty minutes, then cooled

‡ Calculated on the assumption that the copper content of the original skimmilk was 0.12 mgm. per liter.

Knowing of the important rôle of oxygen in copper solution, it was decided to conduct preliminary experiments seeking the explanation for the above phenomena in the dissolved oxygen.

The previously boiled milk was saturated with oxygen by bubbling oxygen through the milk during the entire exposure to copper, and the amount of copper dissolved was compared with that dissolved by raw milk similarly treated. The results, given in table 4, samples *a* to *d*, were unexpected; even when saturated with oxygen, the previously boiled milk dissolved less copper than raw milk. Similar results (samples *e* to *f*) were obtained when air was used instead of oxygen.

TABLE 4

The effect of dissolved gases on the copper solubility in preheated milk

SAMPLE	TYPE OF MILK	TREATMENT OF MILK	TEMPERATURE	COPPER CONTENT OF MILK			COPPER DISSOLVED IN MGM / DM² HR *
				Determination		Mean	
				1	2		
			°C	*mgm /l*	*mgm /l*	*mgm /l*	
a	Raw	Oxygen	23.0	1.658	1.499	1.597	1.38
b	Raw	Oxygen	62.8	11.630	12.850	12.090	11.70
c	Previously boiled	Oxygen	23.0	1.051	1.164	1.108	0.93
d	Previously boiled	Oxygen	62.8	9.970	9.490	9.760	9.30
e	Raw	Air	28.5	0.307	0.302	0.304	0.15
f	Raw	Air	62.8	3.940	3.980	3.960	3.69
g	Previously boiled	Air	28.5	0.403	0.365	0.384	0.23
h	Previously boiled	Air	62.8	2.870	2.810	2.840	2.58

Experimental details:
Surface of copper: 25.8 cm.²
Time of exposure: two hours.
Amount of milk used: 500 cc.
Amount of milk ashed: 250 cc.

* Calculated, assuming that the copper content of the original milk was 0.15 mgm. per liter.

Other experiments of a similar nature were then conducted but using preheating temperatures of 60, 70 80 and 90°C. for one hour. The heating was done in the quart milk bottles which were to be used later for the solubility tests. During the preheating the milk was agitated by means of the same device used in the solubility tests but with glass slides in place of metal test pieces. After the heating and holding at the desired temperature for one hour, the milk was promptly cooled to the desired temperature by running cold water into the water bath. The results of the various experiments are given in table 5.

In the first series of samples, the milk was cooled to 60°C. and held at that temperature for ten minutes, the copper test pieces were then substituted for the glass slides, and agitation continued for two hours at 60°C. The results again show a reduced copper solubility when the milk had been preheated to temperatures above 70°C. It will be noted in this series of samples as in the following series, that milk preheated at 70°C. for one hour

TABLE 5

The solubility of copper in preheated milk

SERIES	SAMPLE	HEAT TREATMENT	TREATMENT DURING EXPOSURE	TEMPERATURE	COPPER CONTENT OF MILK IN MGM /L	COPPER DISSOLVED IN MGM / DM ²/HR
		°C		°C.		
1	a	60 (1 hour)	None	60	4 340	4 05
	b	70 (1 hour)	None	60	4 868	4 56
	c	80 (1 hour)	None	60	2 750	2 52
	d	90 (1 hour)	None	60	3 555	3 30
2	e	60 (1 hour)	Bubbling oxygen	23 5	1 841	1 64
	f	70 (1 hour)	Bubbling oxygen	23 5	2 445	2 22
	g	80 (1 hour)	Bubbling oxygen	23 5	1 937	1 73
	h	90 (1 hour)	Bubbling oxygen	23 5	2 095	1 88
3	i	90 (2 minutes)	Bubbling oxygen	24 5	1 928	1 72
	j	90 (20 minutes)	Bubbling oxygen	24 5	1 959	1 75
	k	90 (40 minutes)	Bubbling oxygen	24 5	2 252	2 04
	l	90 (60 minutes)	Bubbling oxygen	24 5	1 944	1 74
4	m	60 (1 hour)	Carbon dioxide	60	0 874	0 70
	n	70 (1 hour)	Carbon dioxide	60	0 701	0 53
	o	80 (1 hour)	Carbon dioxide	60	0 535	0 37
	p	90 (1 hour)	Carbon dioxide	60	0 419	0 26

Experimental details:

Surface of copper: 25 8 cm.^{2}

Time of exposure: Two hours.

Amount of milk used: 500 cc. (whole milk).

Calculated on the assumption that the copper content of the original milk was 0.15 mgm. per liter. The milk used in each experimental set was taken from the same lot of milk, so that the results are not influenced by variations in the composition of the milk.

dissolved more copper than milk preheated at 60°C. These results appear to parallel the increased copper solubility in pasteurized milk, observed by Miscall et al. (7).

In the second series of samples, the milk was cooled to 23.5°C. and held at that temperature for ten minutes; the copper test pieces were then substituted for the glass slices and the bubbling of oxygen through the milk was started; agitation, the bubbling of oxygen and exposure to copper were then continued for two hours at that temperature. The results (samples *e* to *f*) indicate that the bubbling of oxygen through the milk tends to eliminate the effect of preheating on the amount of copper dissolved.

In the third series of samples all the milk was preheated to 90°C. using the same procedure as before, but varying the holding time. After the holding time had elapsed, the samples were cooled to 24.5°C. and exposed to copper under the same conditions as in the second series. The results (sample *i* to *l*) lead to a similar conclusion as in the preceding series.

In the fourth series of samples the procedure was the same as in the first series, but bubbling of carbon dioxide through the milk was started when the copper test pieces were placed in the milk. The results (samples *m* to *p*) indicate that the expulsion of dissolved oxygen apparently is not the only effect of the heat treatment. However, this indication is not conclusive, because not sufficient time was allowed for the complete removal of the oxygen by rinsing with carbon dioxide, before the milk was exposed to the copper.

These preliminary experiments indicate that the effect of preheating on copper solubility must be sought in the gaseous content of the milk. A comparison of samples *m* to *p* with samples *a* to *d* demonstrates the effects of the removal of dissolved oxygen on copper solubility. It should be noted that even at 60°C. the copper solubility in samples *m* to *p* is less than would normally be expected at room temperature.

If the dissolved gases affect copper solubility, they probably exert this effect through their influence on the oxidation intensity which can be measured in terms of oxidation-reduction potentials. It was therefore decided to undertake experiments attempting to correlate oxidation-reduction potentials of the milk under various conditions to copper solubility.

6. The solubility of copper in relation to the oxidation-reduction potential

The solutions of metals, however complicated the mechanism of solution may be, fundamentally involves the change of the reduced form of the metal to an oxidized form, or a change of the free energy of the metal. Oxidation may be produced by chemical agents, or by electrochemical means. The effect of atmospheric oxygen in metal solution is commonly appreciated, but in addition the literature on metal corrosion gives reports of agents other than oxygen accelerating metal solution, such as ferric ions, cupric ions and chlorine.

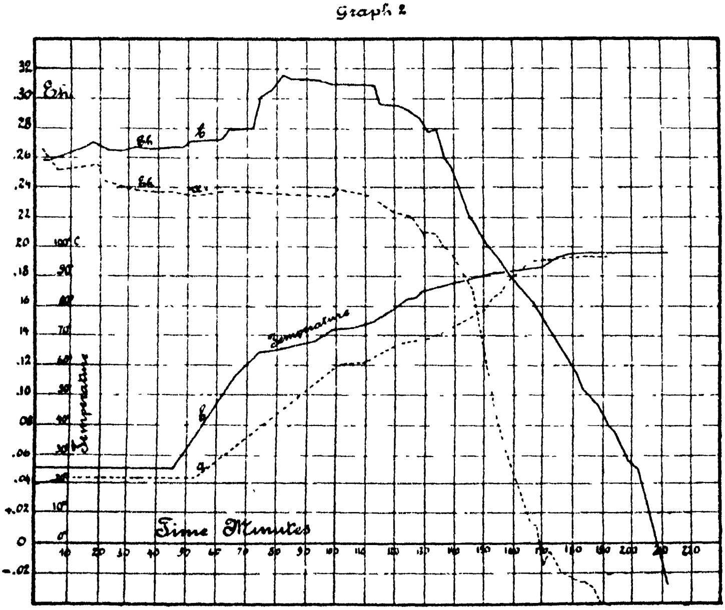
The conception of metal solution as an oxidation process is not new, but few attempts have been made to correlate the rapidity of metal corrosion to the oxidative power of corrosion media, and no attempts have been made to correlate corrosion to oxidation-reduction potentials.

THE PROCEDURE USED IN DETERMINING THE OXIDATION-REDUCTION POTENTIALS

For the measurement of the oxidation-reduction potentials, freshly gold plated platinum electrodes were used. The milk was connected to a M/10 calomel half-cell by means of a saturated potassium chloride-agar bridge. The potential between the gold plated platinum electrode and the terminal of the calomel half-cell was measured by means of a Leeds Northrup, type K, potentiometer. The temperature of the calomel cell was constant within 24° to 26°C., and in each case the temperature was noted and proper corrections applied in the calculation of the E_h.

By means of the oxidation-reduction potential measurements it was hoped to find whether the peculiar temperature-solubility curve for copper in milk could be explained in terms of oxidation intensity. An attempt to measure the oxidation-reduction potential in milk at varying temperatures up to boiling temperature, may at first appear erroneous, because of the wide difference in temperature between the unknown and the calomel half-cell. The use of a saturated potassium chloride salt bridge should greatly minimize the error that might result from a secondary, diffusion potential. Further, the object was merely to obtain comparative potential measurements. For this purpose the procedure gave concordant results.

In the first of these experiments the milk, contained in the quart bottles was heated gradually by raising the temperature of the water-bath. The changes in the potential in the milk, measured at frequent intervals together with the temperature of the milk, are illustrated in figure 3. Because the rate of temperature increase was not uniform, both temperature and potentials are plotted against time; direct plotting of potentials against temperature would be simpler but would not present the facts as

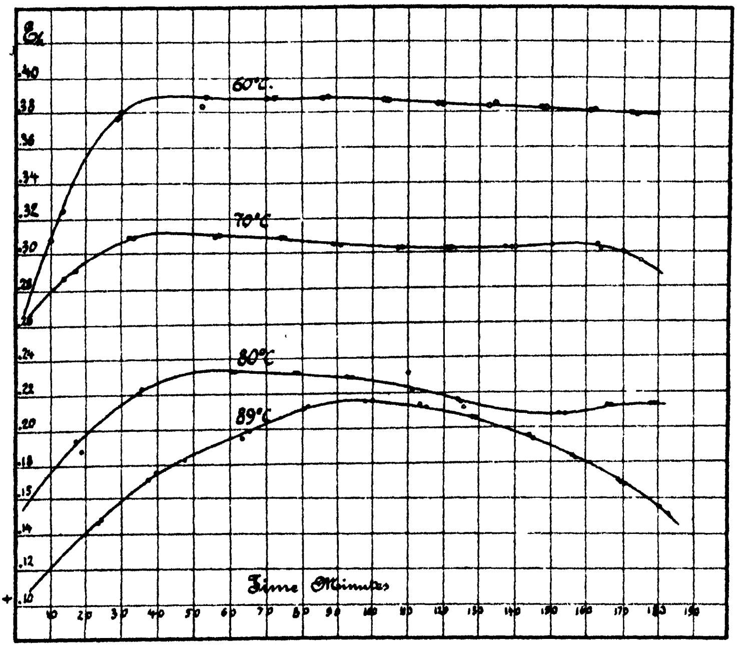


Oxidation-Reduction Potential of Milk in Relation to Temperature & Time
a. agitated by glass slide, b agitated by copper blass

FIG. 3

accurately. The relation between potentials and temperature can be seen by comparing the two on the same "time" ordinate. In one case (curve *a*) the milk was agitated with a glass slide, in the other (curve *b*) with a copper test piece attached to the usual agitating device. From this and similar experiments the following conclusions may be drawn:

Graph 3



The Effect of Temperature on the Oxidation-Reduction Potential in Milk
(when agitated with copper blades.)

FIG. 4

1. Up to a temperature of about 65°C., the oxidation-reduction potential remains substantially constant with increases in the temperature (curve *a*).

2. When the milk is agitated by a copper blade, the potential was found to increase rapidly after seventy-two minutes when the temperature was about 63°C. Upon further increase of the

temperature up to 75°C., the potential remained almost constant.

3. At temperatures above 70°C., (or 75°C. in case of agitating with a copper blade) the oxidation-reduction potential of milk decreases rapidly, and finally reaches negative values somewhat after boiling temperature has been reached.

4. The presence of dissolved copper renders the potential more positive (compare curves *a* and *b*).

TABLE 6

The solubility of copper in milk in relation to the oxidation-reduction potential

SAMPLE	TEMPERATURE DURING EXPOSURE	TIME OF EXPOSURE	GAS TREATMENT	POTENTIAL, E_h		COPPER IN MILK, MEAN OF 2 DETERMINATIONS IN MGM./L.	COPPER DISSOLVED IN MGM./DM.²/HR.*
				Maximum	Final		
	°C.	*minutes*					
a	60	173	None	0.390	0.379	9.02	5.97
b	70	173 130†	None	0.311	0.295	8.17	5.39
c	80	173	None	0.224	0.203	5.50	3.60
d	90	173	None	0.206	0.140	5.24	3.42
				Initial	Final		
e	24.5	120	None	0.271	0.274	0.871	0.70
f	25.5	120	Carbon dioxide	0.159	0.039	0.368	0.21
g	25.5	120	Air	0.287	0.308	1.325	1.14
h	28.0	120	Oxygen	0.331	0.455	1.164	0.98

Experimental details:

Surface of copper: 25.8 cm.²

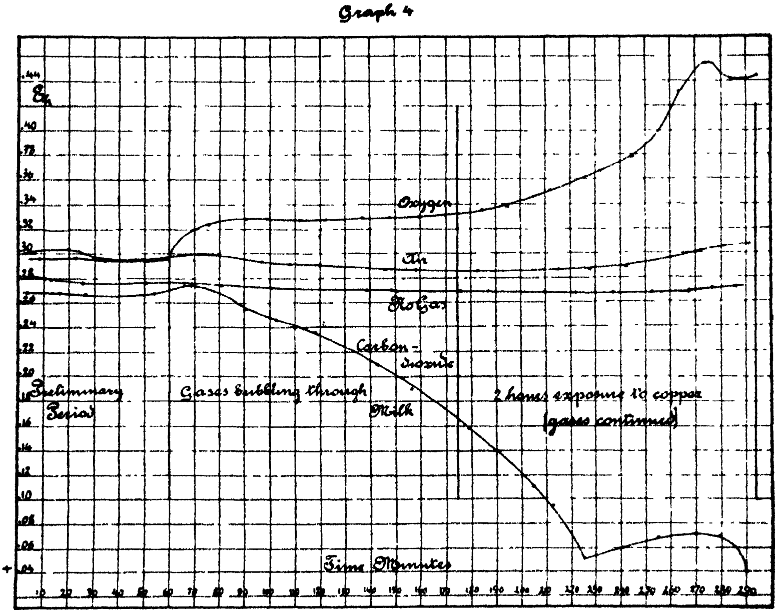
Amount of milk used: 500 cc.

Amount of milk ashed: 250 cc. for samples a–d; 500 cc. for samples e–h.

* Calculated under the assumption that the copper content of the original milk was 0.15 mgm. per liter.

† After one hundred thirty minutes the stirring cable broke; the milk of this sample was not agitated for the remainder of the experimental period.

Experiments of this type, in which the temperature of the milk was raised gradually, the potential was apparently dependent on both, the temperature, and the time. In order to determine the rôle of time, various temperatures were kept constant in the following experiments, and the potential changes with time were observed. (See figure 4.) The finding that the oxidation-reduction potential decreases markedly at temperatures beyond 70°C., seemed a promising indication that this might be an important factor in explaining the marked decrease in copper solubility in milk beyond that temperature. Further experiments were therefore undertaken to study the oxidation-reduction potential of milk at high temperatures and to determine whether there is a relation between the potential and the amount of copper dissolved. The results of such experiments are presented in table 6 and in figures 4 and 5. The details of the experiments are apparent from the description of the general procedure in measuring the potentials, and from the details indicated in the table and graphs. The following conclusions seem justified:



The Effect of Dissolved Gases on the Oxidation-Reduction Potential in Milk

FIG. 5

1. At 60 and 70°C. almost constant potentials are obtained.
2. The amount of copper dissolved is related to the oxidation-reduction potential. The potential attained and the amount of

copper dissolved when oxygen was bubbled through the milk presents an exception to this conclusion, but in this case it was very evident that the copper was coated with an oxide film which undoubtedly acted as a protective coating.

3. Dissolved copper makes the potential more positive, as indicated in figure 4, especially in the sample where oxygen was bubbled through.

DISCUSSION OF EXPERIMENTAL RESULTS

The experiments reported above have concerned themselves with (*a*) acidity, (*b*) dissolved gases, (*c*) temperature, (*d*) preheating time and temperature, and (*e*) oxidation-reduction potential, as factors in determining the amount of copper dissolved by milk. It is now of interest to correlate the isolated conclusions, which were drawn at the end of each experiment, in an attempt to gain a more comprehensive and fundamental understanding of the problem.

The data reported here show that an increased acidity causes a decrease in the solubility of copper in milk. This is contrary to the general belief and previous statements. Rice and Miscall (12) concluded: "Milk slightly sour dissolves but little more copper than normal milk." However, they performed only two experiments and in one of them the amount of copper dissolved in milk was actually less at the higher acidity. This finding that increased acidity causes a decreased copper solubility in milk is in harmony with the conclusions reported by Speller (13) as to the relative importance of the factors that are involved in the corrosion of steel in water within the range pH 4.3 to 10; he enumerates the factors in the order of their importance as follows:

1. Amount of dissolved oxygen present at the metal surface.
2. Protective coatings.
3. Hydrogen-ion concentration.
4. Composition of the metal.
5. Hydrogen overvoltage.

The importance of reaction is subordinate to the effect of dissolved oxygen and protective coatings. As will be indicated later, dissolved oxygen exerts its effect through its influence on the oxidation-reduction potential. It is known that an increase in acidity causes the oxidation-reduction potential to become more negative, and it is probable that the decreased copper solubility with increased acidity is caused by this effect.

The experiments on the effect of oxygen, air and carbon dioxide on copper solubility confirmed, in a general way, the findings of previous workers. The quantitative relationship of the effect of these gases necessarily was different, because of the improved experimental method of exposing copper to milk. The effect of the dissolved gases on the oxidation-reduction potential indicates that they influence copper solubility through their influence on the oxidation-reduction potential. Oxygen affects the oxidation-reduction potential directly; the bubbling of oxygen, air, and carbon dioxide through the milk affects copper solubility only in so far as such a procedure determines the potential by the amount of oxygen remaining dissolved in the milk.

In the experiments on the effect of temperature it was found that copper solubility increased, attained a maximum value, and then decreased markedly with increases in temperature. This agrees, in general, with the findings of Quam (9) and Miscall et al. (7), but in the detailed results a number of differences are found. These differences, as well as the general temperature-solubility relationship, can now be explained.

The increase in copper solubility with temperature is expected and up to 50°C. follows the temperature-reaction-velocity equation. The oxidation-reduction potential remains substantially constant up to this temperature, and apparently is not a limiting factor. At temperatures somewhat above 50°C. the rate of copper solution is such that the oxidation-reduction potential becomes a limiting factor thereby causing a deviation from the temperature-reaction-velocity curve. In the experiments here reported, it was found that the oxidation-reduction potential of milk decreases markedly when milk is heated to temperatures above 70°C. As a result, the oxidation-reduction potential becomes a limiting factor to such an extent that copper solubility actually decreases with further increases of the temperature.

The temperature of maximum solubility reported by Quam was higher (85°C. to 90°C.) than that found above. From the present work it appears that such a high temperature of maximum solubility was to be expected in connection with their experimental procedure where the milk was exposed to the copper in the form of a thin film. Under such conditions the amount of oxygen dissolved in the milk in contact with the copper was obviously higher than what it would have been in the main body of the milk. The high temperature of maximum solubility was therefore not caused by the shorter time of exposure as used in their tests (thirty minutes as compared to one-hundred and twenty minutes), and the lower temperature of maximum solubility reported by others was not due to chemical changes which would supposedly occur in milk when heated to such temperatures for times longer than thirty minutes, as Quam et al. suggested. The same line of reasoning as above also explains the higher copper solubility which was observed by these authors at boiling temperature. On the other hand, the lower temperatures of maximum solubility reported by Miscall et al. were also to be expected because in their experiments no agitation was provided to keep the gaseous composition of the milk uniform and constant during the experiments.

The fact that the temperature of maximum solubility was always found to be 70°C., and that in previous work with other methods different temperatures of maximum solubility were found, indicates that this temperature is largely dependent upon the factors which determine the rate of oxygen diffusion to the metal surface (agitation, aeration, etc.). The procedure which was used in this work in arriving at the conclusion that the temperature of maximum solubility of copper in milk is at 70°C., controlled these essential factors; at the same time these conditions simulate conditions that obtain in cases of submerged corrosion under practical conditions.

The observations on copper solubility reported by Miscall et al. were difficult to explain, but in addition they suggested that the factors which condition copper solution in milk are exceedingly complex. The fact that they found a temperature of maximum solubility not only in raw milk, but also in evacuated milk, or in milk saturated with oxygen, or with carbon dioxide, and especially the fact that in pasteurized milk a maximum solubility is obtained at about the same temperature as is obtained with raw milk, led these authors to conclude that oxygen can not account wholly for the copper solubility in milk, and that there is a reaction over which oxygen has no control. The inconsistencies and lack of correlation in their results are in all probability due to (*a*) lack of agitation in their procedure, (*b*) uncertainty in the amount of oxygen remaining in the milk as the result of the various treatments of the milk, referred to above. The length of time that is required to change the oxidation-reduction potential by passing carbon dioxide through the milk, suggests that more time is required for the removal of oxygen from milk, than is generally assumed.

It has been suggested that an oxygen transmitting enzyme is active in copper solution in milk, and that the break in the temperature-solubility curve at 70°C. and the reduced solubility in preheated milk is due to the destruction of this enzyme. In view of the experimental work presented here, there is no justification for such an assumption, and the above phenomena can be explained on the basis of physico-chemical factors. The observation reported in table 5 (samples *m* to *p*), that the bubbling of carbon dioxide through milk which had been preheated to various temperatures, did not entirely eliminate differences in copper solubility, may be explained by the slow action of carbon dioxide in changing the oxidation-reduction potential, as shown in figure 4. This indicates that the main effect of preheating is the gradual expulsion of dissolved oxygen, thus lowering the oxidation-reduction potential.

In addition to the above, chemical changes may constitute a second factor, involved in the decreased copper solubility in preheated milk. The drop in the oxidation-reduction potential with prolonged heating at 80 to 89°C. (fig. 4) suggests that organic milk constituents undergo chemical changes. It is known that lactose undergoes oxidation in milk at high temperatures (Whittier and Benton (15); this constitutes a mode of oxygen removal that supplements the expulsion of this gas on account of decreased solubility at higher temperatures. Besides the removal of oxygen by chemical union and expulsion from solution, a drop in the potential may be caused by chemical changes that produce substances with reducing properties; for example, it is known that hydrogen sulphide is evolved when milk is heated at high temperatures. Bubbling oxygen through preheated milk will restore the depleted oxygen content, but the effects of certain chemical changes, probably of the type indicated above, are apparently not so readily eliminated. This would account for the decreased copper solubility in previously boiled milk, even when oxygen is bubbled through it (table 4).

In view of the important rôle of oxygen in the solution of copper in milk, analyses to determine the amount of dissolved oxygen under various conditions may appear important. However, no such analyses were made in this work. While they would be of interest, they would not necessarily furnish precise proof of the relationship of dissolved oxygen to copper solubility. The effect of oxygen is operative through its influence on the oxidation intensity which is conditioned by other factors besides the absolute amount of oxygen dissolved. Therefore, the direct measurement of the oxidizing intensity by means of the oxidation-reduction potential was used instead of gas analyses.

The various aspects of the problem as revealed in the present study lead to the conclusion that the solution of copper in milk actually represents a case of metal corrosion in dilute acid solution. The relative importance of the factors that apply in such cases, as enumerated by Speller (13), appear to apply to the present problem. This becomes even more convincing as we compare the copper solubility in milk with the corrosion of metals in other media. Even in strong acid solutions the rate of metal solution is dependent upon the oxygen content of the liquid, as shown by Whitmann and Russell (14). These investigators determined the rates of copper solution in media in equilibrium with (*a*) pure oxygen, and (*b*) air. The solubility with oxygen exceeded that obtained with air, in various media at 22°C. by the following multiples:

6 per cent sulphuric acid	3 7
15 per cent sulphuric acid	3 8
20 per cent sulphuric acid	3 8
6 per cent acetic acid	5 1
6 per cent hydrochloric acid	10.7

They attributed the high solubility ratio in hydrochloric acid to the increased oxidizing capacity of the solution caused by the accumulation of cupric ions.

In this connection it is of interest to note that the ratio of copper solubility in milk when in equilibrium with oxygen to that in equilibrium with air was found as follows:

2.1 at room temperature (table 4, sample *a*, and figure 2 at 20°C.).
2.9 at pasteurizing temperature (table 2, sample *k*, and figure 1, curve *d*, at pasteurizing temperature).
3.1 at pasteurizing temperature (table 4, samples *b* and *f*).

This lower ratio is indicative of the poising effect, or of the slower rate of oxygen diffusion in the milk, which is apparently more pronounced at lower temperatures.

Beyond the scope of this study, the observations on the change of the oxidation-reduction potential in milk are of general interest. Oxidative processes in dairy products are known to influence their flavor and keeping quality, and studies to bring out the relationship of oxidation-reduction potentials to oxidative changes, appear promising.

SUMMARY AND CONCLUSIONS

1. In the review of previous work, the lack of concordant results was attributed to faulty procedure,—lack of agitation, and inadequate control of the temperature and dissolved gases. A procedure was developed to overcome these objections.

2. The effect of acidity, dissolved gases, and temperature on the solubility of copper in milk were studied. The effect of velocity was not studied, but it was controlled and kept uniform in all the experiments.

3. Higher acidities markedly decreased (rather than increased) copper solubility in milk, while slight increases in acidity had no effect.

4. The effect of dissolved gases was studied by bubbling them through the milk during the entire exposure. It was found that carbon dioxide markedly decreased, air slightly increased, and oxygen markedly increased the copper solubility in milk. Prolonged treatment of the milk with oxygen previous to exposure led to the formation of a protective oxide coating.

5. Temperature greatly affected copper solution in milk. The maximum solubility was always found at 70°C. whether the time of exposure was thirty minutes, one hour or two hours. In high acid milk the maximum was also at 70°C., but was appreciably lower than in sweet milk. At boiling temperature copper solubility was about the same as at room temperature.

6. Up to 50°C., the temperature-copper-solubility curve followed the temperature-reaction-velocity curve. At higher temperatures, the curves diverged, less than the expected amount of copper being dissolved.

7. Preheating of milk to various temperatures above 70°C. decreased copper solubility in such milk. The effect of preheating was greater as the temperature and time of preheating increased.

8. The oxidation-reduction potential of milk was found to be related to the solution rate of copper and the various factors that affect it.

9. Up to a temperature of about 65°C., the oxidation-reduction potential remained substantially constant; above 70°C. it decreased rapidly. With prolonged holding at 60 and 70°C., the potential remained constant. The presence of dissolved copper made the potential more positive. Increased acidity is known to make the potential more negative.

10. In the discussion it is shown how the observations on the oxidation-reduction potential may explain the mechanism through which acidity, dissolved gases, temperature and preheating affects copper solubility.

11. Copper solution in milk is a typical case of metal corrosion. The same factors in the same relative order of importance apply to copper corrosion by milk as apply in general to metal corrosion by weakly acid or alkaline solutions.

REFERENCES

(1) Donauer, M.: The action of metals on milk. Ice Cream Review, v, 78, 1922.

(2) Evans, U. R.: The passivity of metals and its relation to problems of corrosion. The Amer. Inst. of Minn. and Met. Eng., Tech. Publ. No. 205, 1929.

(3) Frazer, O. B. J., Ackermann, D. E., and Sands, J.: Controllable variables in the quantitative study of submerged corrosion of metals. Ind. Eng. Chem , xix, 332, 1927.

(4) Gebhardt, H. T., and Sommer, H. H.: Determination of copper in dairy products. Ind. Eng. Chem. Analyt. Ed , iii, 24, 1931.

(5) Guthrie, E. S.: Unpublished paper.

(6) Hunziker, O. F., Cordes, W. A., and Nissen, B. H.: Metals in dairy equipment. Metallic corrosion in milk-products and its effect on flavor. Jour. Dairy Sci., xii, 140, 1929.

Hunziker, O. F.: Selection of metals in the construction of dairy equipment. Proc. World's Dairy Congr., ii, 1189, 1923.

(7) Miscall, O., Cavanaugh, G. W., and Carodemos, C P : Copper in dairy products and its solution under various conditions II. Jour Dairy Sci., xii, 379, 1929.

(8) North, G. C.: Report on chemical and electrochemical tests on metals. For the Creamery Package Co , 1927.

(9) Quam, N. G.: Solubility of metals in milk. Ind. Eng Chem., xxi, 703, 1929.

(10) Quam, N. G.: A mechanical agitator. Ind. Eng. Chem., xx, 908, 1928.

(11) Quam, N. G., Solomon, E. I., and Hellwig, A.: Influence of temperature on the rate of corrosion of copper by milk. Ind. Eng. Chem., xx, 930, 1928.

(12) Rice, F. E., and Miscall, J.: Copper in dairy products and its solution under various conditions. I. Jour. Dairy Sci., vi, 261, 1923.

(13) Speller, F. H : A study of the corrosion factors, and the electrochemical theory. Ind. Eng. Chem., xvii, 339, 1925.

(14) Whitman, W. G., and Russell, R. P.: The acid corrosion of metals; effect of oxygen and velocity. Ind. Eng Chem., xvii, 348, 1925.

(15) Whittier, E. O., and Benton, Anne G.: The formation of acid in milk by heating. Jour. Dairy Sci., x, 126, 1227.

THE RELATIVE ANTINEURITIC (VITAMIN "B") POTENCY OF CERTAIN MILK DERIVATIVES*

G. C. SUPPLEE, O. J. KAHLENBERG, G. E. FLANIGAN AND O D DOW

The Dry Milk Company Research Laboratories, Bainbridge, New York

Although the multiple character of the "vitamin 'B' complex" is now clearly recognized, the lack of universally accepted methods for general assay purposes has retarded the accumulation of data showing the relative distribution of the different factors in various foods. The published investigations however, clearly indicate a relative abundance of vitamin "B"—the antineuritic factor in cereal grains, and somewhat less in animal products including milk, whereas, the reverse is true in regard to vitamin "G"—the anti-pellagric or growth-promoting factor. Various investigations (1-4) have indicated a relatively low vitamin "B" content of milk, and it may be concluded from the data available that this is the limiting factor causing retarded growth in rats when limited amounts of milk are fed as the sole source of this vitamin. Aside from the effect of vitamin "B" on growth, its specificity for curing or preventing polyneuritis in pigeons, rats and man is generally recognized and much of the available data concerning the distribution of this vitamin have been obtained by suitable technique for the cure and prevention of this disease.

EXPERIMENTAL

A comprehensive study of the nutritive properties of a water-soluble milk vitamin concentrate, concerning which a brief preliminary report has been published (5), has necessitated, as a pre-requisite to further studies, a determination of its antineuritic potency. The results of these determinations as well as those obtained from refined lactose, dry whey and rice polishings are recorded herein. The curative technique with the use of pigeons

* Received for publication March 20, 1931.

as recommended by Kinnersley, Peters and Reader (6) (7) was used.

The test substances were rice polishings of commercial grade, milk sugar refined to a degree well beyond the requirements of the U. S. Pharmacopeia standards, whey resulting from a rennet precipitation of casein, subsequently dried by a spray process commonly used in Europe, and the vitamin concentrate having a gross composition computed to the dry basis as follows: Lactose, 52.01 per cent; ash, 30.84 per cent; and nitrogen, 1.54 per cent.

The pigeons used for test purposes were kept on a complete standardized ration for a period of thirty days before placing on the restricted diet prescribed for the development of polyneuritis. This complete diet consisted of 16.6 parts whole buckwheat; 16.6 parts whole cracked corn; 16.6 parts millet; and 50 parts whole wheat. At the end of the thirty-day period on this ration the birds were confined in wire cages with screened bottoms in a warm room in the laboratory building. A restricted ration consisting only of autoclaved polished rice was given ad libitum with 1 cc. of cod liver oil fed weekly by pipette. Those birds which did not develop characteristic opisthotonos or head retraction and convulsions within thirty-five days on the polished rice diet were not used for assay purposes. Many of the birds subjected to the restricted diet developed symptoms between the thirty-fifth and forty-second day, but since these were usually in a weakened condition the reactions from the test substances were irregular and inconsistent. About 5 per cent of the pigeons developed non-typical symptoms, emprosthotonos before the thirty-third day. The results from these non-typical cases were neither consistent nor satisfactory and after preliminary trials with such specimens these cases were discarded. Only 40 per cent of the total number of pigeons given the rice diet were available for test purposes as determined by the development of typical polyneuritis symptoms within the prescribed time. With but few exceptions the afflicted birds were given the second dose of test substance if recovery resulted from the first dose. After the second dosage and at the third onset of convulsions they were given a liberal dose of rice polishings and a yeast tablet consisting of about 250 mgm. and then returned to the complete stock ration for recuperation and recovery.

The test substances were given in limited amounts of water by pipette in all cases within one-half to three hours after the appearance of unquestionable symptoms. When the substance was sufficiently potent recovery was rapid, and with the exception of border-line doses, subsidence of the convulsions and head retraction was brought about within five hours. In many such cases the birds were able to stand erect and consume food and water. In those instances wherein only border-line doses were administered recovery was never complete. In these cases severity of the convulsions usually subsided, head retractions became less pronounced, but the birds continued to lose weight. Very few of these cases recovered to a degree which permitted voluntary food consumption. Forced feeding was resorted to in a few instances, but in all such cases death ultimately ensued with or without re-occurrence of convulsions and opisthotonos.

The records from typical cases wherein reaction time and consistency of results were most regular are shown in table 1. Plate 1 shows the typical reaction of pigeon 46A afflicted with polyneuritis on the nineteenth day. Plate 2 shows the same pigeon five hours after receiving the first 2-gram dose of the water-soluble milk vitamin concentrate. Protection was afforded for one hundred twenty hours by this dosage. The records show that 0.5 gram of rice polishings and 2 grams of the water-soluble milk vitamin concentrate contained substantially the same amount of the antineuritic vitamin. Inconsistencies in the period of protection conferred by the milk vitamin concentrate and dry whey when computed to the day dose requirement are apparent, and it may be considered that while the different levels of these substances exhibited definite antineuritic properties, the more valid basis for computing relative potency must be on those quantities which consistently gave the longest period of protection. A minimum protective period of three days is considered necessary by some investigators (6) (7) (8). Such a basis of appraisal would indicate that 1 gram of rice polishings contained about four times the antineuritic potency of 1 gram of the dry matter of

TABLE 1

Antineuritic potency of test substances as determined by the curative technique with pigeons

PIGEON NUMBER	REACTION TIME	TEST SUBSTANCE	AMOUNT OF TEST SUBSTANCE	PROTECTION PERIOD		REMARKS
				After first reaction	After second reaction	
	days		*grams*	*hours*	*hours*	
23	19	Lactose	0 5	None		No indication of curative effect
46	19	Lactose	0.5	None		No indication of curative effect
83	27	Rice polishings	0 5	110		Improvement within 5 hours—cured
89	31	Rice polishings	0 5	51	48	Improvement within 5 hours—cured
59	32	Rice polishings	0 5	144		Improvement within 5 hours—cured; died after second reaction
61	35	Rice polishings	0 5	144		Improvement after 5 hours—cured; died after second reaction
7615	28	Rice polishings	0 5	140	144	Improvement within 5 hours—cured
741	25	Milk vitamin concentrate	0 75			Only slight improvement—cure not effected
700	33	Milk vitamin concentrate	0 75			Only slight improvement—cure not effected; died after 56 hours
716	33	Milk vitamin concentrate	0 75			Only slight improvement—cure not effected; died after 74 hours
712	28	Milk vitamin concentrate	0 75			Mild reaction—only slight improvement; died after 7 days
69	24	Milk vitamin concentrate	1 50	56	48	Improvement within 5 hours—cured
1056	24	Milk vitamin concentrate	1 50	50	55	Improvement within 5 hours—cured
781	25	Milk vitamin concentrate	1 50	70	96	Improvement within 5 hours—cured

23A	19	Milk vitamin concentrate	2 00	120		Improvement within 5 hours—cured
46A	19	Milk vitamin concentrate	2 00	120	120	Improvement within 5 hours—cured
8	18	Milk vitamin concentrate	2 00	120		Improvement within 5 hours—cured
23B	18	Milk vitamin concentrate	2 00	112		Improvement within 5 hours—cured
55	15	Dry whey	1 50	38	76	Improvement within 5 hours—remained very weak
1056A	22	Dry whey	1 50	73	27	Improvement within 5 hours—remained very weak
46B	24	Dry whey	1 50	26	20	Improvement within 5 hours—remained very weak
69A	21	Dry whey	1 50	62	35	Improvement within 5 hours—cured
713	26	Dry whey	1 50	97	42	Improvement within 5 hours—cured
1345	26	Dry whey	1 50	96		Improvement within 5 hours—not completely cured
1342	32	Dry whey	3 00	88	24	Improvement within 5 hours—cured
1346	33	Dry whey	3 00	48	45	Improvement within 5 hours—cured
1355	34	Dry whey	3 00	72		Improvement within 5 hours—cured
1056B	18	Dry whey	3 00	102	47	Improvement within 5 hours—cured
69B	22	Dry whey	3 00	65	52	Improvement within 5 hours—cured
55A	15	Dry whey	3 00	76		Improvement within 5 hours—cured

the water-soluble milk vitamin concentrate, and eight or slightly more times that of the same amount of dry whey.

A preliminary report (5) on the growth-promoting properties of the water-soluble milk vitamin concentrate indicated that slightly more than 1 gram per day of this substance was required to supply the vitamin "B" complex requirement for the normal growth of white rats. It will be shown in a subsequent report that the limiting factor in these earlier studies was vitamin "B" and not vitamin "G." Other investigators (3) (9) have shown that 3 to 3.5 grams of milk solids per day is required to supply the vitamin "B" requirement of the growing rat. A previous communication (10) which came to us recently stated that a sample of the water-soluble milk vitamin concentrate, now under investigation at another laboratory, contains about four-tenths as much vitamin "B" as a good grade of yeast. This assay was made by the pigeon maintenance technique with a sample of the concentrate from which about 40 per cent of the inert material contained in the product reported in this paper had been removed.

SUMMARY

The vitamin "B" assay of a water-soluble milk vitamin concentrate from which the fat, casein, albumen and a substantial proportion of the milk sugar had been removed showed an antineuritic potency substantially 25 per cent of that of rice polishings, according to the curative technique with pigeons.

Desiccated whey solids, assayed according to the curative technique with pigeons showed an antineuritic potency of not more than 10 to 12 per cent of that of rice polishings, or slightly less than one-half that of the water-soluble milk vitamin concentrate.

REFERENCES

(1) Daniels, A. L., and Brooks, L.: Further evidence of destruction of vitamin "B" in evaporated milk. Proc. Soc. Exp. Biol. and Med., 1927, xxv, 161.

(2) Macy, I. G., Outhouse, J., Graham A., and Long N.: Human milk studies. III. The quantitative estimation of vitamin "B." Jour. Biol. Chem., 1927, lxxiii, 189.

(3) Outhouse, J., Macy, I. G., Brekke, V., and Graham, A.: A note on the vitamin "A" and "B" content of cows' milk. Jour. Biol. Chem., 1927, lxxiii, 203.

(4) Hunt, C. H., and Krause, W. E.: The relative antineuritic and antipellagric potency of cows' milk. Jour. Biol. Chem., 1928, lxxix, 733.

(5) Supplee, G. C., Dow, O. D., and Flanigan, G. E.: The concentrated water-soluble fraction of milk as a source of vitamin "B." Jour. Dairy Sci., 1928, xi, 420.

(6) Kinnersley, H. W., and Peters, R. A.: Antineuritic yeast concentrates. IV. The further purification of yeast vitamin "B." Biochem. Jour., 1928, xxii, 419.

(7) Kinnersley, H. W., Peters, R. A., and Reader, V.: Antineuritic yeast concentrates. III. The curative pigeon test: A critique. Biochem. Jour; 1928, xxii, 276.

(8) Guha, B. C., and Drummond, J. C.: XCVII. Observations on the Concentration of Vitamin "B." Biochem. Jour., 1929, xxiii, 880.

(9) Supplee, G. C., Dow, O. D., and Nelson, J. W.: Richesse en Vitamines du lait liquide et du lait sec. Le Lait, 1927, vii, 12.

(10) Personal communication from Dr. George R. Cowgill, Yale University, New Haven, Connecticut.

PLATE 1

Pigeon 46A with Polyneuritis Reaction after Nineteen Days on Polished Rice Diet

PLATE 2

Pigeon 46A Five Hours after Receiving a 2-gram Dose of the Water-soluble Vitamin Concentrate of Milk

Characteristic polyneuritis symptoms have disappeared; protection lasted for five days.

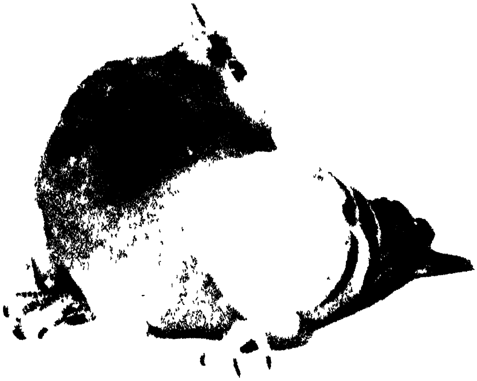


PLATE 1

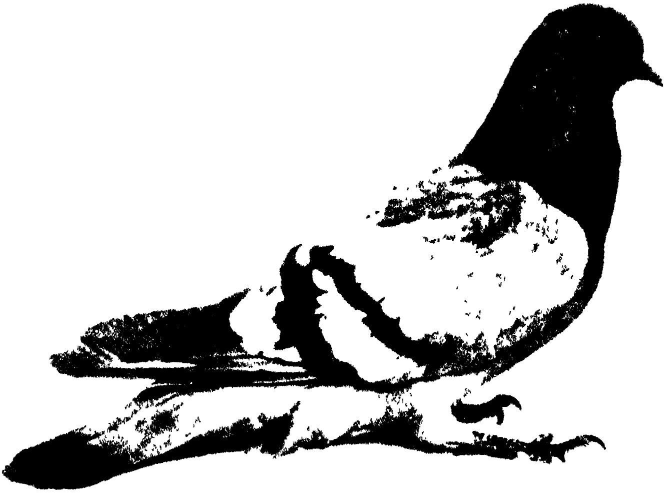


PLATE 2

MALT FLAVOR IN RAW MILK PRODUCED BY MICROCOCCUS*

P. H. TRACY AND R. J. RAMSEY

Department of Dairy Husbandry, University of Illinois, Urbana, Illinois

During the past summer (1930) a malt-like flavor was detected in some of the raw milk delivered at the University Creamery. So objectionable was this flavor that some of the affected milk could not be used for market milk purposes. Pasteurization did not remove the defect entirely.

As the acidity of this milk usually was not over 0.18 per cent the flavor was first attributed to feed the cows had eaten. A survey of the pasture and feed lot on one of the farms sending in the malt flavored milk, however, failed to reveal any weeds or other feeds that might possibly cause the defect. The milk was stored over night in cans in an outside galvanized iron tank away from feed odors. It was found that the first milk through the milking machine developed much more of the off-flavor. It was also noted that the night's milk only was affected at the time of delivery to the plant at about 9 a.m. The milking machine, according to the farmer's statements, was washed once a day and was rinsed with a sodium hypochlorite solution before using. Examination of the machine showed the rubber parts to be in rather poor condition, being full of small crevices on the inside. Upon replacing these tubes with new ones, the flavor disappeared for a few days but later returned suggesting the possibility of bacteria as being the cause of the malt flavor.

EXPERIMENTAL

A malt flavor in milk and butter was studied by Hammer and Baker (1) and found to be caused by *Streptococcus lactis var. maltigenes*. They report the flavor and odor as being very apparent in certain raw milks and creams entering dairy plants at acidities under 0.2 per cent. They also state that the defect was found to be more evident in summer than winter. The

---

* Received for publication March 23, 1931.

optimum temperature for growth of the bacteria and development of the flavor was given as 37°C.

In our studies an organism (culture 20) was isolated from some of the malt-flavored milk, which, when grown in pure culture at 68°F., reproduced the flavor defect to some extent. This organism is not the same as the one reported by Hammer and Baker, being a micrococcus of aureus type.[1] It was noted that in all the milk that had a pronounced malt flavor a spore-forming organism of the *B. subtilis* group was commonly present (culture 1). Although culture 20 alone will produce a slight malt flavor, the presence of culture 1 seems necessary for a distinct flavor development, indicating an associative action between the two orgaisms. It may be that enzymes produced by culture 1 make it possible for certain proteolytic actions of culture 20 to progress more rapidly. This was evidenced by the fact that the addition of pepsin and rennet to separate inoculations of culture 20 resulted in a decrease in time required for the malt flavor to appear.

It was observed that the most pronounced malt flavor occurred in those raw milks in which there was a predominance of cultures 1 and 20. The flavor was not so evident when acid-forming bacteria were present. It was also noticed that in some instances a strong malt flavor would be present, but would later disappear after the milk stood at room temperature for a few hours.

OCCURRENCE

Plant men in various parts of the country during the past summer and fall have complained of a feed-flavor in raw milk which, in some cases, they attributed to the cows eating weeds, because of the shortage of grass pasture. Examination of some such milk by one of the authors revealed the fact that the flavor was identical to the malt-flavor of bacterial origin. A study made at a central Illinois dairy showed that the milk delivered by twenty of the three hundred producers had the malt-flavor. Organisms similar to culture 20 in appearance and in ability to produce malt flavors have been isolated from the milk delivered by twenty of the twenty-two University milk patrons. However, the milk from only three of these patrons has been a serious source of the flavor.

---

[1] A culture of *S. lactic var. maltigenes* secured by the authors from Hammer when grown in pure culture produced a very potent malt flavor identical to the one produced by the micrococcus.

Malt-flavor producing micrococcus organisms were found in aseptically drawn milk from the udders of twenty-four apparently healthy cows on three farms producing milk containing the off-flavor. The companion organism (culture 1) was found to be rather commonly distributed on utensils and in the soil of the barnyards.

TABLE 1

Description of cultural characteristics of organisms producing malt-like flavor in milk

	CULTURE 20	CULTURE 1 (SUBTILIS GROUP)
Classification	Micrococci	Bacillus
Form—Arrangement	Packets—coccus	Long rods in chains
Sporulation	−	+
Motility	−	+
Color	Orange	Dirty white
Occurrence	Udder	Hay, soil
Colony formation	Small punctiform 1 mm.	Flat spreading 6 mm.
H_2S from peptone	−	−
Nitrites from peptone	−	−
Nitrites from nitrates	+	+
Starch hydrolysis	−	+
Litmus milk	Very slow acid curd	Slightly acid rennet curd
Dextrose	Acid, no gas	Acid, no gas
Lactose	Acid, no gas	Acid, no gas
Sucrose	Acid, no gas	Acid, no gas
Glycerol	Acid, no gas	Neutral, no gas

CULTURAL CHARACTERISTICS

The cultural characteristics of the two organisms (1 and 20) responsible for the malt-flavor in milk are given in table 1.

When a purified casein solution was inoculated with cultures 1 and 20, malt-like odors were obtained suggesting a protelytic action. In methylene blue milk, reduction took place in one instance in two hours and fifteen minutes with a count of 700,000 and in another case in one hour and forty-five minutes with a count of 1,600,000.

TABLE 2

Effect of pasteurization upon culture 20

Sample	*Bacteria per cc.*
Raw	8,400,000
Heated to 122°F.	6,200,000
" " 131°F.	5,600,000
" " 140°F.	4,800,000
" " 142°F.	3,000,000
Held at 142°F. for 2 minutes	1,056,000
" " 142°F. " 5 "	420,000
" " 142°F. " 10 "	150,000
" " 142°F. " 15 "	1,000
" " 142°F. " 20 "	No colonies

TABLE 3

Relation of temperature to the development of malt-like flavor in milk

TEMPERATURE	COUNT AFTER FOUR HOURS' INCUBATION	TIME WHEN FLAVOR FIRST DETECTED	COUNT WHEN PRONOUNCED FLAVOR FIRST DETECTED
°F.	*per cc*		*per cc.*
60	(1) 50,000 (20) 630,000	No malt-like flavor even after 72 hours (cheesy odor)	3 days total count of 15,000,000
68	(1) 180,000 (20) 680,000	20 hours	(1) 920,000 (20) 4,800,000
85	(1) 111,000 (20) 920,000	9 hours	(1) 880,000 (20) 9,500,000
100	(1) 150,000 (20) 1,420,000	6 hours	(1) 800,000 (20) 9,000,000

THERMAL DEATH POINT (ABSOLUTE)

That a micrococcus causing a malt flavor in milk may be responsible for diarrhea and other intestinal disturbances was suggested by Ramsey and Tracy (2). In view of the fact that our data suggest that this type of organism may be of rather common occurrence, it seems that its resistance to pasteurizing temperatures would be of importance from the public health standpoint. As shown by data in table 2, the organism is destroyed by ordinary pasteurizing procedures. Culture 1, however, was found to survive pasteurization and was not completely destroyed even at boiling temperatures.

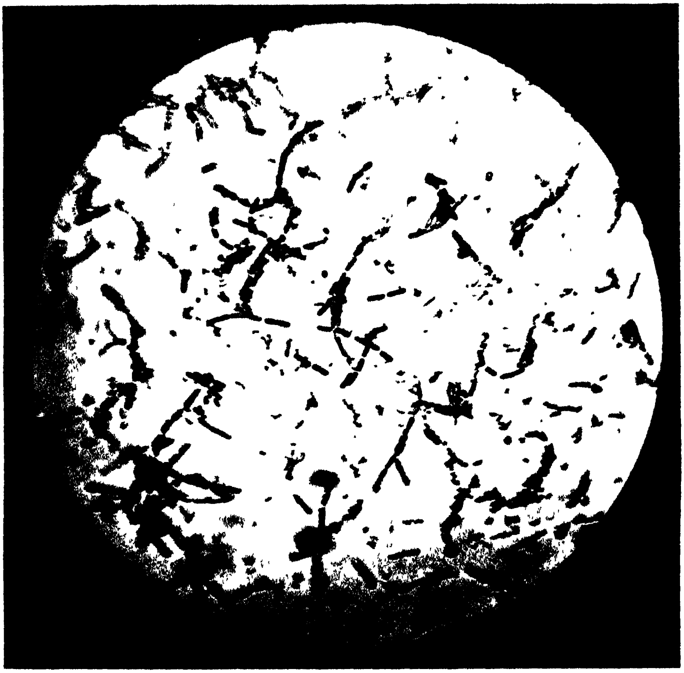


FIG. 1. PHOTOMICROGRAPH OF CULTURE 20 (MICROCOCCUS) AND CULTURE 1 (BACILLUS) WHICH WERE FOUND TO PRODUCE A MALT FLAVOR IN MILK

Organism 20 will produce the flavor when grown in pure culture but when culture 1 is present a much more pronounced malt flavor results. (Magnification 1130.)

IMPORTANCE OF TEMPERATURE IN DEVELOPMENT OF MALT FLAVOR

In studying the occurrence of the flavor in raw milk delivered at the plant, it was noted that the defect was most common when the nights were warm. Table 3 shows the relation existing between temperature and the development of the flavor in milk.

Although the defect occurred sooner in samples held at the higher temperatures (85° to 100°F.), the most characteristic flavor was in the milk held at 68°F. It is interesting to note that the flavor failed to develop in the milk held at 60°F. even after seventy-two hours with a count of 15,000,000, which probably explains why the flavor is most common in summer months.

SUMMARY AND CONCLUSIONS

Data have been presented to show that an objectionable malt-flavor in milk may be caused by the action of a micrococcus of aureus type (culture 20). It was also found that when an organism of the *B. subtilis* group (culture 1) was also present, a much more pronounced malt-flavor resulted. The presence of acid-forming bacteria retarded the development of the flavor.

The flavor defect was found to be rather common in raw milks entering certain milk plants during the summer months. Not all the samples containing the suspected organisms had the flavor at the time they arrived at the plant. In some instances, the flavor disappeared from samples held at room temperature.

Utensils were found to be a direct source of the large rod (culture 1), while the coccus forms (culture 20) were found in the udders of cows in suspected herds.

Neither the coccus or the rod-shaped organisms were found to produce gas. They were proteolytic in their action. Pasteurization of pure cultures of the coccus organisms (culture 20) at 142°F. resulted in their death after twenty minutes, whereas, the rod-shaped organisms (culture 1) were found to survive even higher temperatures.

The malt flavor was found to develop most rapidly in milk held at 85°F. and 100°F. although a more characteristic flavor was noted in the milk held at 68°F. The defect failed to develop in milk held at 60°F. for three days.

REFERENCES

(1) HAMMER, B. W., AND BAKER, M. P.: Classification of the *Streptococcus lactis* group. Research Bulletin No. 99. November, 1926. Agr. Exper. Sta. Iowa State College and Mechanic Arts, Ames, Iowa.

(2) RAMSEY, R. J., AND TRACY, P. H.: Food poisoning probably caused by orange colored Staphylococus from udders of apparently healthy cows. Proc. Soc. Exper. Biol. Med., 1931, xxviii, 390–391.

A STUDY ON METHODS FOR DETERMINING NUMBERS OF MOULDS AND YEASTS IN BUTTER*

I. THE RELATION OF THE pH OF THE MEDIUM

A. H. WHITE AND E. G. HOOD

Division of Dairy Research, Department of Agriculture, Ottawa, Canada

The rapid development in the use of the routine mould and yeast analysis of butter as an index of the sanitary conditions and the efficiency of manufacturing processes in the creamery make it desirable that uniform procedures be adopted if the results from different laboratories are to be comparable. In Canada, uniform methods for mould and yeast analysis are especially necessary due to provincial mould and yeast competitions and to the adoption of the modified score card (1) for exhibition butter.

The importance of adopting uniform procedures for the microbiological analysis of butter was recognized by the American Dairy Science Association and the report (2) of the sub-committee studying this question has been published recently.

In a study of the media commonly used by various workers in analyzing butter for moulds and yeasts, Hood and White (3) found that one of the most variable factors was the final pH of the media which ranged from 3.4 to 4.6. When the pH value of the medium was higher than 3.8 numerous bacterial colonies were present on the plates which increased the total number of colonies greatly, made counting difficult, and introduced inaccuracies in the yeast count. From the results of this study the authors recommended a final pH of 3.5 ± in the medium for Canadian laboratories.

Clerkin (4) also found that as the pH value of the medium varied from 3.5 to 6.0 the total number of colonies of bacteria and yeasts increased but that the ratio of bacteria to yeasts increased with the higher pH values. This author concludes that

---

* Received for publication March 24, 1931.

the medium used must have a pH of 3.5 ± before the plate counts obtained could, with safety, be regarded as consisting entirely of yeasts.

Hammer (5) states that 1 cc. of 1 per cent tartaric acid solution per plate (8 to 10 cc. medium) gives satisfactory results but does not limit the growth of bacterial colonies, and that with greater amounts of acid there is a gradual decrease in the number of yeasts developing.

The divergence of opinion among the various workers, as evidenced by the final pH of the medium they employed, suggested that a study be made of the relation of the pH of the medium, firstly, to the growth of pure cultures of yeasts isolated from butter, and secondly, to yeast counts as obtained from butter itself.

METHODS

For the first part of the study, 63 cultures of yeasts were isolated from numerous samples of commercial and exhibition butter representative of all parts of Canada. The yeasts were isolated from plates poured with a malt agar medium pH 5.5 ± and picked to malt agar slopes. For purification purposes the cultures were then transferred to beer wort broth and high dilutions of the broth culture were plated using malt agar. Well isolated yeast colonies were transferred again to malt agar slopes at pH 5.5 from which further transfers were made during the investigation.

Before plating, the yeasts were grown in malt extract broth pH 4.8 ± for twenty-four hours at 25°C. at which time all cultures had produced good growth. Suitable dilutions were then made by transferring one loopful of the twenty-four-hour malt broth culture to a sterile 99 cc. water blank, and 1 cc. of this dilution was used for plating.

For the second part of the investigation 50 samples of butter were analyzed and included both pasteurized and unpasteurized butter representative of different grades according to Canadian standards. A preliminary mould and yeast count of the butter was made and then a dilution of either 1:10, 1:100, or 1:1000 was used for plating according to the preliminary count.

Both the pure yeast cultures and butter samples were plated on dehydrated malt agar (Difco) as this medium had been previously recommended by Hood and White (3) in their tentative methods for mould and yeast analysis. The medium was acidified to pH values of 3.0, 3.4, 3.8, 4.2 and 4.6 just before plating with a 5 per cent solution of lactic acid (U. S. P. 85 per cent). A LaMotte colorimetric hydrogen ion testing set was used to standardize the pH of the medium using bromphenol blue as an indicator. These pH values were selected because they represented the range of the final pH of the different media used by various workers.

For both yeast cultures and butter samples, plates were poured in duplicate for each pH value of the medium and incubated at 25°C. for five days. Counting was done with the naked eye and the results expressed as the average counts of the duplicate plates.

Before counting, the plates from the butter samples were carefully examined with a good hand lens to pick out if possible any bacterial colonies. Only colonies that appeared to be yeasts were counted and recorded. After counting, a microscopic examination of stained smears of 12 colonies from each plate was made. These colonies were picked from a representative area of one plate of each sample, and were typical of all colonies that were counted in this area. When bacterial colonies were found, the ratio of bacterial colonies to yeast colonies was considered to be representative of the whole plate and the counts were corrected accordingly.

RESULTS FROM PLATING PURE CULTURES OF YEASTS

In table 1 the average counts of duplicate plates for each pH value of the 63 yeast cultures used in this investigation are tabulated.

The results in table 1 show that the number of colonies from individual yeast cultures growing at the different pH values of the medium compare favourably with one another when the limitations of the plate method are taken into consideration. For cultures 15, 24, 27, 55 and 62 there was a noticeable decrease

TABLE 1

Counts of yeast cultures on media of different pH values

NUMBER OF CULTURE	pH 3.0	pH 3.4	pH 3.8	pH 4.2	pH 4.6
1	159	157	169	186	167
2	230	206	234	206	231
3	18	18	20	17	19
4	386	362	346	364	350
5	246	210	227	216	219
6	225	232	222	251	237
7	454	343	424	385	389
8	201	212	189	203	205
10	296	352	338	310	318
11	452	487	493	404	434
13	168	181	171	179	176
15	8	16	17	23	19
17	68	77	71	80	80
18	63	73	64	62	71
19	93	107	119	111	108
20	236	232	206	223	224
21	244	248	296	270	272
22	136	136	147	119	127
23	110	123	130	127	136
24	64	129	166	186	180
25	55	47	60	68	68
26	41	38	42	39	48
27	1	26	23*	28	27
28	256	281	277	295	275
29	81	67*	75	86	84
30	644	662	641	654	670*
31	155	165	153	147	142
32	39	41	39	40	33
33	134	172	129	142	135
34	168	174	202	187	173
35	400	337	340	363	360
36	128	128	138	145	137
37	180	199	193	182	203
38	226	199	211	193	193
39	320	303	343	300	311
40	339	337	364	319	315
41	332	333	343	328	336
42	248	240	228	235	249
43	342	384	384	407	334
44	268	227	253	234	214
46	130	132	147	165	146
47	385	381	397	443	418
48	403	447	425	465	439

TABLE 1—*Concluded*

NUMBER OF CULTURE	pH 3.0	pH 3.4	pH 3.8	pH 4.2	pH 4.6
49	388	413	401	401	374
50	345*	361	389	353	333
51	346	354	357	348	335
52	222	212	228	252	231
53	315	342	330	312	317
54	200	190	182	174	164
55	177	584	588	573	582
56	360	416	387	420	409
57	254	231	251	257	236
58	704	707	736	702	722
59	629	692	651	656	651
60	106	118	98	125	105
61	437	441	476	444	453
62	0	327	323	335	303
63	137	139	119	125	165
64	662	626	663	664	640
65	199	202	209	228	212
66	330	325	326	327	318
67	713	689	707	647	673
69	200	196	212	213	213
Average of all cultures	252	266	271	269	265

* Counts for one plate only.

in the number of colonies on the medium with a pH of 3.0 but there was no significant difference in the counts of these cultures at pH 3.4 to 4.6, and for the remaining cultures there was no great variation in the counts at the different pH values.

The average counts at each pH of the medium for all cultures did not show any significant differences; the highest average count of 271 was obtained at a pH of 3.8 and the lowest average count of 252 was for a pH of 3.0, a difference of only 19 colonies. The difference between the average counts at pH values of 3.4 to 4.6 was only 6 colonies.

The variation between the high and low average counts obtained at the different pH values was only 7 per cent, while for individual cultures the greatest variation in the counts at pH 3.4 to 4.6 was 30.7 per cent for culture 24, and the smallest variation was 2.8 per cent for sample 66. For 25 cultures there was a difference of less than 10 per cent between the high and low counts, and for 55, or 87 per cent, of the cultures the difference did not exceed 20 per cent.

The results also show that the high and low counts of individual cultures were obtained at all pH values of the medium. Ten of the cultures had high counts even when the medium had a pH of 3.0, while the low counts for 20 of the cultures were obtained at this pH value. However, as none of the media commonly used in analyzing butter for moulds and yeasts is acidified to pH 3.0, the counts obtained at this pH are not considered further but are of interest in that they indicate that many of the yeasts commonly found in butter are able to grow and develop at a pH value lower than is commonly used for mould and yeast control work.

TABLE 2

Showing the distribution of the high and low counts of individual cultures according to the pH value of the medium

pH OF MEDIUM	NUMBER OF CULTURES WITH HIGHEST COUNTS	NUMBER OF CULTURES WITH LOWEST COUNTS
3 4	12	21
3.8	20	14
4.2	22	12
4 6	6	13
Total cultures .. .	60	60

In table 2 a summary is given showing the distribution of the high and low counts for pH values of 3.4 to 4.6.

In addition to the 60 cultures summarized above, the high counts for three cultures were the same at pH values of 4.2 and 4.6, and the low counts for three cultures were the same at two different pH values.

These figures clearly show the even distribution of the high and low counts from the individual yeast cultures over the different pH values, and indicate that the yeasts used in this study were able to grow equally well over the range of pH studied.

After counting, all plates were examined carefully to determine if the pH of the medium had any effect on the size of the colonies. In the case of ten cultures, representative surface colonies were measured and there was found to be no appreciable difference in the size at any pH value. To the eye, the subsurface colonies of some yeast cultures seemed to grow slightly larger at pH values of 3.8 to 4.6 than at pH values of 3.0 and 3.4, but with the majority of the cultures there was no apparent difference in the size of subsurface colonies.

There was a good development of the yeasts on all plates after five days at 25°C. and all colonies were discerned easily with the naked eye.

RESULTS FROM PLATING BUTTER SAMPLES

In table 3 the numbers of yeast colonies from butter samples are recorded which appeared on the medium at different pH values.

A study of the data presented in table 3 shows that for the majority of the samples there were no marked differences in the number of yeast colonies that developed at pH values of 3.4 to 4.6, and in many cases the counts at pH 3.0 were comparable to the other counts. These results also show that the maximum and minimum counts for individual butter samples occurred at all pH values of the medium, excluding the counts at pH 3.0 which is considerably below the range of pH of the media commonly used for routine yeast and mould analysis of butter.

One sample had the highest count at a pH of 3.4, 8 samples at a pH of 3.8, 19 at a pH of 4.2, and 22 at a pH of 4.6. The minimum counts were also found at every pH as follows: 33 samples at a pH of 3.4, 8 at a pH of 3.8, 2 at a pH of 4.2, and 5 at a pH of 4.6, with one sample having the same count at pH values of 3.4 and 4.6, and another at pH values of 3.4, 3.8 and 4.2.

While maximum and minimum counts were found at all pH values of the medium, the general trend of the results showed that the number of yeast colonies decreased slightly as the pH of the medium increased. This is more clearly shown in the average number of yeast colonies and the average estimated yeast counts for each pH value of the medium as summarized in table 3.

TABLE 3

The number of yeast colonies from butter samples for each pH value of the medium

SAMPLE NUMBER	DILUTION	pH 3.0	pH 3.4	pH 3.8	pH 4.2	pH 4.6
1	1:1,000	19.0	35.5	36.5	45.5	59.0
2	1:100	142.5	156.5	179.0	189.0	183.0
3	1:100	343.5	393.5	425.0	411.5	455.0
4	1:100	13.0	17.5	21.0	17.0	13.5
5	1:1,000	44.0	51.0	55.5	63.5	67.0
6	1:1,000	131.0	141.0	148.5	144.0	135.5
7	1:10	69.5	122.0	170.0	185.5	180.5
10	1:100	95.0	81.0	69.0	83.5	99.0
11	1:10	7.0	6.5	9.5	13.0	12.5
12	1:10	5.5	7.0	9.0	11.5	10.5
13	1:10	2.5	4.5	4.5	4.5	5.0
14	1:100	126.0	151.5	179.5	174.5	195.0
15	1:100	11.0	18.0	26.5	34.0	31.0
16	1:100	20.0	21.5	32.0	35.0	31.0
17	1:100	59.0	56.0	66.5	85.0	81.5
18	1:100	18.5	21.5	25.0	31.0	35.0
19	1:100	17.5	15.5	21.0	16.0	23.0
20	1:10	132.5	133.0	133.5	147.0	124.5
21	1:10	10.0	24.5	24.0	29.0	34.0
22	1:10	2.0	4.0	6.5	4.5	6.0
23	1:10	286.0	410.5	440.5	462.0	445.5
24	1:100	47.5	65.0	72.0	69.5	68.0
25	1:100	35.0	47.5	66.0	62.0	53.0
26	1:10	7.0	13.0	22.0	27.0	30.5
27	1:10	17.0	46.0	42.5	39.0	52.0
28	1:10	14.5	23.5	36.5	38.0	39.5
29	1:10	48.0	45.0	71.0	83.5	73.0
30	1:1,000	236.5	217.5	241.5	252.5	291.5
31	1:100	13.5	35.5	61.5	71.5	74.5
32	1:100	60.0	76.5	77.0	85.0	84.0
33	1:10	19.5	21.5	20.0	22.0	26.0
34	1:10	165.0	187.0	179.5	189.0	186.5
35	1:100	38.5	41.5	59.5	52.5	49.5
36	1:100	49.0	44.0	58.0	61.5	67.0
37	1:100	34.5	34.0	33.0	46.5	55.5
38	1:100	13.0	28.0	28.5	32.0	28.0
39	1:100	24.0	28.0	30.5	34.0	31.0
40	1:10	52.0	67.0	90.0	102.5	118.5
41	1:100	587.5	692.0	692.5	692.0	671.5
42	1:10	207.5	227.5	252.5	235.5	258.5
43	1:10	193.5	302.0	288.5	307.5	290.5
46	1:100	262.0	280.5	298.5	297.0	283.5
47	1:1,000	39.0	45.0	54.0	66.5	63.0

TABLE 3—*Concluded*

SAMPLE NUMBER	DILUTION	pH 3.0	pH 3.4	pH 3.8	pH 4.2	pH 4.6
48	1:1,000	9.5	16.5	17.5	15.0	20.5
49	1:100	33.0	32.0	30.5	34.0	30.0
50	1:100	96.0	90.0	113.5	117.0	108.0
52	1:100	4.0	6.0	7.0	14.0	11.5
53	1:100	112.0	132.5	121.0	130.0	124.0
54	1:100	375.5	403.5	453.0	438.5	471.0
55	1:100	31.5	41.5	40.0	45.5	53.5
Averages 50 samples		87.6	103.2	112.8	116.9	118.8
Estimated average yeast count		15,153	16,480	18,003	18,838	19,930

Although these figures show lower average yeast counts at the lower pH values of the medium, the variations in the counts at pH values of 3.4 to 4.6 are not great enough to be of practical significance in routine analysis of butter. The maximum average number of colonies per plate at pH 4.6 was only 13.5 per cent greater than the minimum average at a pH of 3.4, and the variation between the maximum and minimum estimated yeast counts per cubic centimeter of butter was approximately 17 per cent.

For individual samples, the greatest percentage difference in the counts was for sample 26, the minimum count being 57.4 per cent lower than the maximum count, while the smallest variation was 3.1 per cent for sample 41. The average variation for all samples was approximately 27 per cent. In general the widest percentage variations in the counts occurred in the case of samples where there were comparatively few colonies appearing on the plates due to using too high dilutions or to samples being moderately low count butter.

In table 4 is shown the distribution of the samples of butter according to the percentage variation in the counts when the medium was acidified to pH values of 3.4 to 4.6.

These figures show that 42 samples, or 84 per cent, had a variation of less than 40 per cent between maximum and minimum counts, with only 8 samples having variations of over 40 per cent.

As previously mentioned in the outline of the methods of this study, all plates from butter samples were examined for bacterial colonies, and a microscopic examination made of stained smears of twelve representative colonies from a selected area of the plate. This examination showed that, in the case of 40 samples of butter, bacterial colonies were easily distinguishable at pH 4.2 and 4.6. Many of the plates were crowded with bacterial colonies, while in others only a few were evident.

With 14 samples of butter some of the subsurface colonies that were counted as yeasts were found to be bacteria on a microscopic examination of the smears. In all cases, with one exception, not more than two bacterial colonies were found in the twelve that were examined. On none of the plates were bacterial colonies evident at pH 3.0, 3.4 or 3.8, nor were any bacteria found on the examination of the smears under the microscope.

TABLE 4

The distribution of butter samples according to the percentage variation in the yeast counts at different pH values

PER CENT VARIATION	NUMBER OF SAMPLES	PER CENT OF SAMPLES
Less than 10	7	14
10–20	11	22
20–30	10	20
30–40	14	28
40–50	4	8
Over 50	4	8
Total samples	50	100

As with the pure yeast cultures all plates were carefully examined to determine if the pH of the medium made any noticeable difference in the size of the yeast colonies. There was no apparent difference in the size of the surface colonies at any pH, and the colonies that were present on the medium of pH 3.0 and 3.4 grew and developed as well as at the higher pH values.

The number of moulds appearing on the plates were very few, many of the samples of butter showing no mould growth at any pH of the medium. For the samples that showed mould growth, there was practically no difference in the numbers developing on the plates at any pH.

DISCUSSION OF RESULTS

From the results obtained in the study of representative yeasts isolated from Canadian pasteurized butter, it was apparent that all cultures were able to grow equally well over a range of pH values from 3.4 to 4.6 which represented the range of pH of the media commonly used for routine mould and yeast analysis of butter, while the majority of samples were able to make good growth even at a pH of 3.0.

These results showed that a pH of approximately 3.5± had no detrimental effect on the development of butter yeasts, and as there was no apparent difference in size of colonies or rapidity of growth, the data indicated that a medium of pH 3.5± would give as satisfactory yeast counts from butter as a medium of pH 4.2 or 4.6 which is commonly used by some workers.

For the butter samples, while the general trend of the results was for the number of yeast colonies to decrease as the pH of the medium increased, the data reported do not show differences in the counts great enough to be considered significant in the routine analysis of butter for moulds and yeasts.

Furthermore, the decrease in the counts was not consistent for individual cultures and maximum and minimum counts were obtained at all pH values, excluding the counts at pH 3.0. The average variation in the counts of individual samples and the difference in the average counts at each pH were less than found by several workers (6, 7, 8) in making a series of plate counts of the same sample of milk and using the same medium. This is also true in regard to the results from pure yeast cultures and would indicate that the variations in the yeast counts as shown by these data were probably due more to technique and personal factors than to the difference in the pH of the medium.

The results also show that when the medium had a pH of 4.2 and 4.6, bacterial colonies were always present on the plates, while at pH 3.0, 3.4, and 3.8 no bacterial colonies were evident although Hood and White (3) in previous work found a few bacteria when media had a pH of 3.8. Even when the plates at pH values of 4.2 and 4.6 were carefully examined with a hand lens, and in some cases with the low power lens of a microscope, some bacterial colonies were counted as yeasts and were only distinguishable by a microscopic examination of stained smears. This fact, no doubt, increased to some extent the counts obtained at pH 4.2 and 4.6.

Such a time consuming practice as the examination of colonies under the low power lens of a micrsocope and of making stained smears of doubtful colonies would be impractical in the control laboratory making a routine analysis for moulds and yeasts of a great many samples of butter per day.

When the medium is acidified to a pH of 3.5± the growth of bacteria on the plates is inhibited and the laboratory worker is reasonably sure that all colonies are yeasts. This not only makes for greater ease and quickness in counting but eliminates the error due to the inclusion of bacterial colonies, which would tend to give greater uniformity to mould and yeast counts when made by different workers. This is important in Canada where results from different laboratories are used for comparative purposes and where such counts are taken into consideration in scoring exhibition butter.

In this connection it is interesting to note that while the yeast counts for butter samples at pH 3.4 and 3.8 were slightly lower in general than at pH 4.2 and 4.6 there was not a sufficient difference to change the rating of the count according to the classification (9) as used in Canada, nor to change the numerical score given for mould and yeast counts by the modified score card (1) for butter. Thus there is no advantage in using a pH of 4.2 or 4.6 for mould and yeast work and there is the decided disadvantage of having bacterial colonies on the plates at the higher pH values which tends to introduce errors in the counts.

SUMMARY AND CONCLUSIONS

The data presented show that for pure cultures of yeasts there was no significant differences in the average counts or the counts of individual cultures at all of the pH values used. Maximum and minimum counts of individual cultures were obtained at all pH values. For butter samples, the data showed that in a general way the yeast counts decreased slightly as the pH of the medium increased but the differences in the counts were insignificant at pH values of 3.4 to 4.6 for routine control work.

At pH values of 3.4 and 3.8 no bacterial colonies were found on the plates. For the majority of the butter samples, however, many bacterial colonies were evident at pH values of 4.2 and 4.6. Although the majority of the bacterial colonies were easily distinguishable some were included in the counts as yeasts even after a careful examination of the plates, which introduced errors in the counts.

The pH of the medium had no apparent effect on the size or rapidity of growth of the yeast cultures from the pure cultures of yeasts and from butter.

While few samples had mould counts high enough to obtain much comparative information, there was no indication that the growth of moulds was affected by the pH values of the medium used in the study.

As the data show that pH values of 3.4 to 3.8 in the medium effectively inhibits the growth of bacterial colonies, but does not affect significantly the mould and yeast counts of butter, a pH of 3.5 ± which allows for a margin of safety in standardizing the medium is considered to be most satisfactory for the routine analysis of butter for moulds and yeasts.

REFERENCES

(1) Hood, E. G., and White, A. H.: Studies on moulds and yeasts in creamery butter. Part III. Pamphlet No. 92 New Series. Dominion of Canada, Department of Agriculture.

(2) Report of sub-committee on microbiological analysis of butter. Jour. Dairy Sci., xiii, No. 5, Sept., 1930.

(3) Hood, E. G., and White, A. H.: Part I, Pamphlet No. 92 New Series. Dominion of Canada, Department of Agriculture.

(4) Clerkin, P.: Estimation of yeasts and moulds in butter. Department of Agriculture Journal, Dublin, xxviii, No. 2.

(5) Hammer, B. W.: Studies on yeasts in dairy products. Jour. Dairy Sci., ix, November, 1926.

(6) Wright, W. H., and Thornton, H. R.: How accurate is the quantitative plate count? Abstract Jour. Bacteriol., xiii, 63, 1927.

(7) Mudge, C. S., and Lawler, B. M.: Is the statistical method applicable to the plate count? Jour. Bacteriol., xv 207, 1928.

(8) Ellenbergar, H. B., Bond, M. C., Robertson, A. H., and Moody, Ruth I.: A comparison of the methylene blue reduction test and the agar plate count for determining the quality of milk. Vermont Agr. Exp. Sta. Bul. 264, February, 1927.

(9) Hood, E. G., and White, A. H.: The cause and prevention of mould in Canadian pasteurized butter. Dom. of Canada, Dept. of Agr. Bulletin No. 48 New Series, 1925.

FURTHER REMARKS ON DATA ON BACTERIAL COUNTS PRESENTED BY BREW AND DOTTERRER AND ANALYZED BY BREW*

G. A. BAKER

Statistician, New York State Department of Health, Albany

Bacterial counts are commonly used by health departments in discriminating against sources of supply of milk for fluid use. It is usual in routine work to use some method of estimating the gross number of bacteria in milk. The number of bacteria, in the same lots of milk, estimated by various methods often varies considerably. Thus it may become of interest to compare the estimates of the number of bacteria in the same lots of milk by two or more different methods.

A comparison of the estimates of the bacteria in raw milk by means of the agar plate method and the direct microscopic method has been made by Brew (1) on the basis of data presented by Brew and Dotterrer (2). It is the purpose of this paper to present a few statistical considerations that were overlooked in the statistical analysis of the data but which serve to strengthen the remarks of Dr. Brew made in the text of his paper and to make a few additional remarks.

The portion of the data of Brew and Dotterrer that is to be discussed here by way of illustration consists of 491[1] comparative bacterial counts by the agar plate method and the direct microscopic method on raw market milk.

The statistical analysis of these data was not sufficient to indicate the true relation between the agar plate and direct micro-

---

* Received for publication March 23, 1931. Acknowledgments are made to Dr. J. D. Brew, Milk Specialist, New York State Department of Health and to Mr. W. D. Tiedeman, Chief of the Bureau of Milk Sanitation, New York State Department of Health for advice in this presentation.

[1] The first 52, the last 7, and the numbers 131 and 357 of these counts are omittted in this presentation.

scopic methods of counting bacteria. It is not necessary with this particular type of data to consider the whole mass of data at one time and to summarize it into a single set of figures. In this case it is better not to do so because the counts vary over such a tremendous range and the extreme items, in summary figures, entirely mask the relation between the counts in the more interesting portions of the ranges and because the marginal distributions are of doubtful significance where the object is merely to compare two methods. The marginal distributions, when comparisons between the estimates of numbers of bacteria by two different methods are being made, depend upon the arbitrary selection of the person collecting the data. The best comparisons would be obtained if, when the plate counts were being compared with the direct microscopic counts, there was about the same number of plate counts for each sub-interval of the direct microscopic counts. That is, the marginal distribution of the direct microscopic counts would be rectangular. Similarly, if the direct microscopic counts were to be compared with the plate counts on a considerable number of different lots of milk the best comparison would be obtained if the marginal distribution of the plate counts was approximately rectangular. With data that can have any shape of marginal distributions depending upon the choice of the investigator it is better to regard the correlation coefficient and other constants as mere summary figures and not to attempt any statement of the limits of random variations unless the number of observations is large. Any set of data could, perhaps, be regarded as a random sample from some population and if the number of observations was very large, probable errors could be calculated on this basis. If probable errors are given based on ordinary formulae it is necessary to show rigorously that the data approximate both in form and in method of collection the assumptions on which the formulae were derived. The data analyzed by Brew do not sufficiently approximate the ordinary type of data that has been dealt with heretofore to make it apparent that the ordinary expressions for random sampling variations apply when the whole mass of data is considered at once.

A much more enlightening statistical procedure than the one employed by Brew would be to block off a certain interval, say from greater than zero to 100,000 count, for the direct microscopic method and to investigate the locus of the mean values of the bacterial counts by the agar plate method as the direct microscopic counts cover this range. This principle is followed in the accompanying figures.

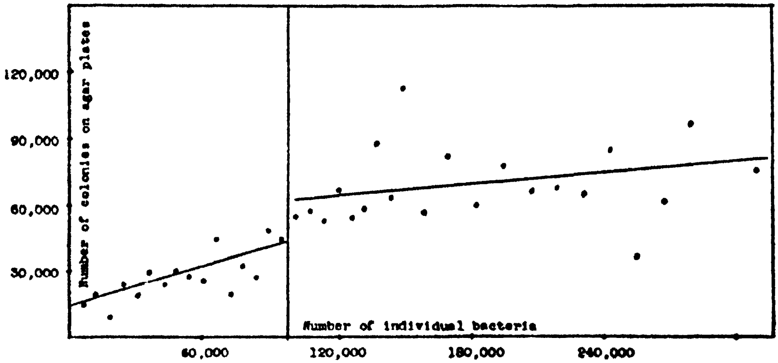


FIG. 1. LOCUS OF MEAN PLATE COUNTS FOR GIVEN DIRECT MICROSCOPIC COUNTS

	Sub-interval I	*Sub-interval II*
Constant (calculated without grouping):		
Mean direct microscopic count	31,500	174,500
Standard deviation direct microscopic count	26,000	53,000
Mean plate count	24,300	68,200
Standard deviation plate count	27,000	49,300
Correlation coefficient	.22	12
Regression equation	Mean plate count = 23 direct microscopic	Mean plate count = .12 direct microscopic

Figure 1 shows the relation between the number of colonies on agar plates and the number of individual bacteria determined by the direct microscopic method for 209 samples of low count milk and for 100 samples of medium count milk. The dots represent actual observed average plate counts for the designated direct microscopic counts. The sloping lines are the lines that "best" represent the locus of the observed means in each sub-interval. If the data were adequate, the locus of the modes or medians of the distributions of the plate counts for given direct microscopic counts might be of great deal more interest because of the great influence of extreme items on the arithmetic mean. It will be seen for these particular data that when the direct microscopic count is in the interval greater than zero to 96,000 the average plate counts increase about one-fifth as fast as the direct microscopic counts. There is an abrupt rise in the locus of the mean plate counts at a direct microscopic count of about 96,000 and then the locus proceeds almost on a level to a direct microscopic count of 315,000. This means that a knowledge of the direct microscopic count in the latter interval is of almost no value in predicting or estimating the plate count.

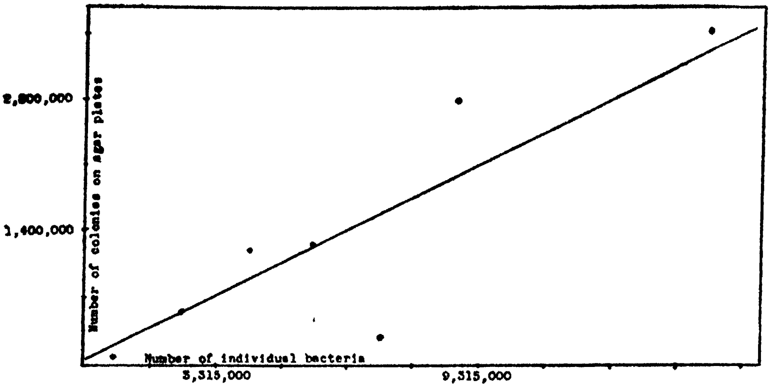


FIG. 2. LOCUS OF MEAN PLATE COUNTS FOR GIVEN DIRECT MICROSCOPIC COUNTS

Constant (calculated without grouping):	
Mean of direct microscopic counts	1,695,000
Standard deviation of microscopic counts	2,042,000
Mean of agar plate counts	424,000
Standard deviation of agar plate counts	722,000
Correlation coefficient	.62
Regression equation	Mean plate count = .22 direct microscopic

Figure 2 shows the relation between the mean number of colonies on agar plates and the number of individual bacteria by the direct microscopic method for 120 samples of high count milk.

The very extreme samples show a much higher association. These 120 items are almost entirely dominated by a few extreme items which would also dominate the whole mass of data if they were included in the summary figures. The inclusion of these extreme items would give a false notion of the degree of association between the two methods. It should be noted that the second figure is drawn to quite different scales than is the first.

The locus of mean direct microscopic counts for given values of the agar plate counts would probably exhibit similar characteristics.

These data are inadequate to warrant definite conclusions, but they suggest:

a. That, if the direct microscopic method really gives an estimate of the number of bacteria, the plate method under-estimates the number of bacteria in almost all milk with a direct microscopic count over 180,000

b. That both methods are subject to considerable errors of estimation, and

c. That it is very desirable to form some notion of the shape and location of the frequency distributions of the errors of estimation of each method as a basis for judging the significance of differences in counts and the adequacy of a certain number of determinations to estimate, within certain limits, the true number of bacteria.

In order to accomplish (*c*) the approach must, necessarily, be empirical. The obvious and direct method of finding out an empirical approximation to the simultaneous variations in the errors of estimation of each of two or more methods would be to run several hundred determinations by each method at the same time on each of a set of samples of milk containing, at least roughly, graduated numbers of bacteria. If determinations by two different methods that are assumed really to give estimates of the number of bacteria in milk are made at the same time on the same sample of milk, the estimates should be compared directly and not some function of them, as the logarithms. This is true because the effect of the increase of bacteria with time is eliminated by making both determinations at the same time.

The question is: How does the number of bacteria estimated by one method compare with the number of bacteria estimated by another method? The location (or mean measured from the true number of bacteria in the milk) of the distributions of the estimates of the number of bacteria can not be determined by this method. The mean numbers of bacteria in each lot of milk as obtained by two methods can be compared and if they differ markedly, then it can be concluded that one or both methods are definitely biased. It must be left to other considerations to show which is essentially the more accurate.

Since the plate method gives higher and then lower counts than the direct microscopic method, it is to be expected that for a certain range the mean counts will be in substantial agreement. Outside of this range the plate method shows a pronounced and progressive bias as compared with the direct microscopic method. The effects of variations in technic and in the types of bacteria present in the milk and of changes in the technician could also be approximated by repeated trials on the same lots of milk.

The foregoing discussion assumes that it is of interest to compare the estimates of the number of bacteria in milk as made by different methods. It may be that the limitations of the various methods and the unique knowledge obtained by each makes it not worth while to attempt a comparison of this kind (3). If, however, such a comparison is attempted and it is assumed that the methods employed actually give estimates of the number of bacteria present in milk, then the statistical methodology would be that outlined above.

REFERENCES

(1) Brew, J. D.: The comparative accuracy of the direct microscopic and agar plate methods in determining numbers of bacteria in milk. Jour. Dairy Sci., July, 1929, xii, no. 4.

(2) Brew, J. D., and Dotterrer, W. D.: The number of bacteria in milk. New York Agr. Expt. Station Bul. 439, Geneva, N. Y., 1917.

(3) Robertson, A. H., and Frayer, J. M.: Variability, accuracy, and adaptability of some common methods of determining the keeping quality of milk. V. The broader aspects of variability. Vermont Agr. Expt. Station Bul. 318.

THE INFLUENCE OF ENVIRONMENTAL TEMPERATURE ON THE PERCENTAGE OF BUTTER FAT IN COW'S MILK*

H. J. BROOKS

Kansas Agricultural Experiment Station, Manhattan

It is generally accepted that there is an inverse correlation between the environmental temperature and the percentage of butter fat in cow's milk. It was with the aim of verifying this under Kansas conditions and specifically stating this relationship that the present study was made.

RÉSUMÉ OF PREVIOUS STUDIES

Eckles (1) states that regardless of when the lactation begins, the percentage of fat plotted followed a curve for the year, lowest in June and July and gradually rising to the highest point in December and January, and then declining again until midsummer. A total of 240 cows was tabulated.

White and Judkins (2) concluded that milk tests lower in fat content in the summer months than it does in the winter months, and that this variation is due to seasonal changes. This conclusion was drawn from data taken on 49 cows over a period of seven and one-half years.

Hills (3) says that from results obtained in a study of the local creamery butterfat tests of the milk of 30 herds in Vermont during the months of May, June and July, 1891, and July, August and September, 1892, "it points strongly to the probability that when cows are put on pasture the percentage of fat rises as the temperature falls, and falls as the temperature rises.

---

* Contribution No. 75 Department of Dairy Husbandry. Acknowledgment is made of the assistance rendered by Professor Salmon of the Department of Agronomy, Mr. Hodgson of the Department of Dairy Husbandry, and Professor Andrews of the Department of Education in the preparation and statistical interpretation of this study. Received for publication March 30, 1931.

In other words, the percentage of fat in milk varies inversely with the temperature changes."

Brooks (4) found that when warming a stable for cows during the months of December, January, February and March, 1895, there was a definitely lower percentage test in the wing that was warmed to 55°F. and maintained at this temperature.

Hays (5) concluded from a study of the University of Missouri dairy herd over a period of 258 days starting in January, in which the environmental temperatures were recorded, that, generally, the lower the temperature the higher the fat test. This variation amounted to 0.079 per cent for each 10° lowering of the temperature. In a second phase of this experiment, Hays kept two Jersey cows in a controlled temperature chamber in which the temperature ranged from 92.7° to 27°F—a spread of 65.7°. This variation was accompanied by a spread of 0.624 per cent test which gives an average of 0.095 per cent for each 10° lowering of the temperature.

Weaver (6) found from a study of the Iowa State College dairy herd over a period of one year that the fat test was highest during the first half of the winter, or during January, gradually declining to the second half of the summer, during August and early September, when the lowest test occurred, and then increasing rather rapidly in the fall. Ayrshire and Holstein tests were approximately 0.6 per cent lower in the second half of summer than in the first half of winter. Guernsey and Jersey tests were approximately 1.1 per cent lower. The butter fat tests were found to be lower with higher outside and inside temperatures.

Clothier (7) and Hooper (8) alone attribute the variation in percentage of fat to factors other than temperature or season of the year. The former finds it "impossible to believe that the season variations in butter fat content of the milk, observed in Arizona, are not due directly to changes in feed," and the latter concluded from a study of 88 Jersey cows, one-fourth of which freshened in each of four seasons, that the fat test runs lower while the cow is fresh, regardless of the time of the year she freshens, and there is no indication that the percentage of fat runs lower in July.

Ragsdale and Turner (9) found in a study of some 4100 Guernsey, Jersey and Holstein-Friesian Advanced Registry records that, irrespective of time of freshening, "the percentage of fat in milk when plotted follows a general curve, being lowest during the summer months, then gradually rising reaching a peak during the winter months and then again declining during the spring and summer." The peak was reached in December at 4.75 per cent and the lowest point in August at 4.32 per cent.

Ragsdale and Brody (10) concluded from a controlled experiment on ten animals over the period from March 1 to May 1 that "There is a relation between temperature and percentage of fat showing roughly an increase of about 0.2 per cent in the fat for a decrease of 10°F. in the temperature between the observed temperature limits." The animals were kept indoors during stormy days and cool nights and the barns were well ventilated though not artificially heated.

PLAN OF PROCEDURE

This study is concerned with the question of the influence of environmental temperature on the percentage of butter fat in cow's milk. Such factors as the stages of lactation and gestation, condition of animal, feeding and breed difference have been eliminated. To accomplish this only records of 365 days duration were used, and these were selected so as to have, as nearly as possible, the same number of animals freshening each month, and the same number of animals from each of the four major dairy breeds. This distribution is shown in table 1.

The data were coded by calendar months so that the animal's monthly production fell in the respective calendar month irrespective of the time of freshening. The totals thus gave the production of the group by the month irrespective of the time of freshening. A total of 409 lactations were taken from the records of the cows in the Kansas Agricultural Experiment Station dairy herd over the period from 1912 to 1927. The mean temperature for each month for this period was taken from the Kansas Weather Bureau Reports for the Manhattan district. In securing the average percentage of butter fat, true averages were determined in each case by dividing the total butter fat production by the total milk production. Milk and butter fat production are expressed in pounds produced each day by the group for the respective months.

TABLE 1

Distribution by breeds of the number of animals freshing each month

BREED	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	TOTAL LACTATIONS
Holstein	9	9	11	9	6	12	9	9	8	11	8	10	111
Ayrshire	9	9	7	10	9	6	10	9	10	7	9	10	105
Jersey	9	9	5	7	8	6	11	9	11	6	9	6	96
Guernsey	9	7	12	6	11	4	3	9	6	11	9	10	97
Total	36	34	35	32	34	28	33	36	35	35	35	36	409

TABLE 2

Variations in the percentage of butter fat with the fluctuation in environmental temperature irrespective of time of freshening

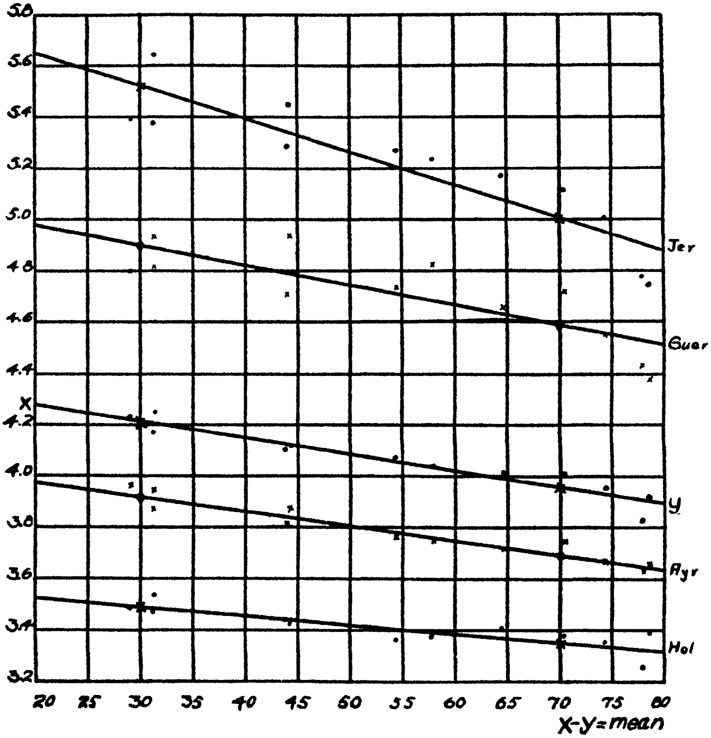
MONTH	TEMPERATURE	AVERAGE TEST	AVERAGE DAILY MILK PRODUCTION	JERSEY	GUERNSEY	AYRSHIRE	HOLSTEIN
	°F.	*per cent*	*pounds*	*per cent*	*per cent*	*per cent*	*per cent*
January	29.0	4.23	23.5	5.39	4.80	3.96	3.49
February	31.2	4.16	24.5	5.38	4.82	3.87	3.47
March	43.9	4.01	25.3	5.28	4.71	3.81	3.42
April	54.4	4.07	26.3	5.27	4.74	3.76	3.37
May	64.5	4.01	26.9	5.18	4.66	3.72	3.41
June	74.3	3.95	25.7	5.01	4.55	3.67	3.36
July	78.5	3.92	23.7	4.75	4.38	3.66	3.39
August	77.9	3.83	24.9	4.78	4.43	3.63	3.26
September	70.4	4.01	24.7	5.12	4.72	3.75	3.38
October	57.7	4.04	24.2	5.24	4.83	3.75	3.38
November	44.1	4.11	23.8	5.45	4.94	3.87	3.43
December	31.3	4.25	23.0	5.64	4.93	3.94	3.54

The seasons were designated as spring, summer, fall and winter consisting of March, April, May; June, July, August; September October, November; December, January, February, respectively.

The influence of environmental temperature on the percentage of butter fat is shown by the data in table 2.

These data show that there is a close inverse correlation between the percentage of butter fat in cow's milk and the environmental temperature. The coeficient of correlation for this relationship was found to be:

$$r = -0.872 \pm 0.046$$



Graph I
Trend in the Percentage of Butter Fat
with Variation in the Environmental Temperature

This relationship is further expressed in figure 1. In this graph the ordinate expresses the percentage of butter fat, and the abscissa the environmental temperature in degrees Fahrenheit. The mean data for all animals is expressed by the line x-y, while that for the respective breeds is shown by the lines: Jer. (Jersey), Guer. (Guernsey), Ayr. (Ayrshire), and Hol. (Holstein).

Line x–y of figure 1 shows that with an increase in environmental temperature there is a uniform decrease in the percentage of butter fat. This relationship assumes a distinctly linear regression which may be expressed by the regression equation:

$$y = 4.41 - 0.0064\ X$$

in which y equals the percentage of butter fat and x equals the temperature in degrees Fahrenheit.

It may be noted from table 2 and figure 1 that the same general relationship exists between the environmental temperature and the percentage of butter fat for each of the four breeds. The more pronounced fluctuations are found in the higher testing breeds, however, with consistent though less marked variations in the lower testing breeds. The irregularities noted within the breeds tend to be smoothed out when the data for all breeds are combined as in line x–y.

TABLE 3

Summary of mean seasonal data irrespective of time of freshening

SEASON	MILK PER DAY	FAT PER DAY	PERCENTAGE OF BUTTER FAT	TEMPERATURE
	pounds	*pounds*		*°F.*
Spring	26.2	1.0637	4.060	54.2
Summer	24.7	0.9631	3.899	76.9
Fall	24.2	0.9806	4.052	57.4
Winter	23.7	0.9990	4.215	30.5

The regression equations for the respective breeds are:

Jersey .... $y = 5.90 - 0.0127\ X$
Guernsey .... $y = 5.14 - 0.0078\ X$
Ayrshire .... $y = 3.59 - 0.0033\ X$
Holstein .... $y = 4.09 - 0.0056\ X$

The data for all animals were summarized by seasons and the means for the seasons were determined and are shown in table 3. This table brings out the similarity between the average temperatures and the percentages of butter fat in the spring and fall seasons as contrasted with the extremes exhibited in the summer and winter periods. It is noted that the lowest mean temperature of 30.5°F. occuring in the winter season is accompanied by the highest average percentage of butter fat, while the highest mean temperature of 76.9°F. in the summer season is coincident with the lowest percentage of butter fat. The spring and fall seasons fall between these extremes in both mean temperature and average percentage of butter fat.

RELATION BETWEEN MILK PRODUCTION AND PERCENTAGE OF BUTTER FAT

It is frequently observed that there is an inverse relationship between the amount of milk produced and the percentage of butter fat in the milk. In order to determine the extent of this relationship in this study the average amount of milk produced daily each month was determined for all cows irrespective of time of freshening and correlated with the respective percentages of butter fat for these months. The coefficient of correlation for this relationship was found to be:

$$r = -0.1675 \pm 0.190$$

This would indicate that the amount of milk produced in the respective months was not a determining factor in the percentage of butter fat obtained for the group within that month when the factor of the stage of lactation was eliminated.

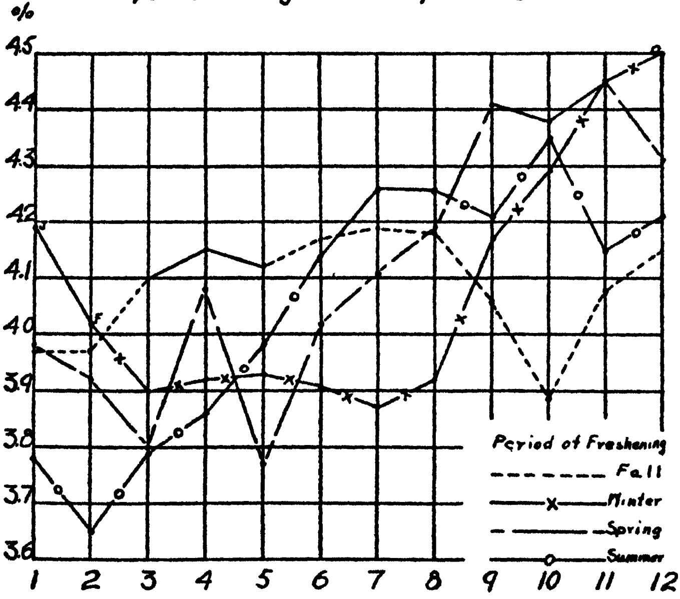
RELATIVE INFLUENCE OF THE ENVIRONMENTAL TEMPERATURE AND THE STAGE OF LACTATION ON THE PERCENTAGE OF BUTTER FAT

Ragsdale and Turner (11) and others have shown that as the normal lactation of a cow advances there is a noticeable decline in the percentage of butter fat from the first month to the second or third months of the lactation followed by a gradual increase in the percentage of butter fat which becomes more pronounced during the last months of lactation.

In view of the definite influence of the environmental temperature on the percentage of butter fat demonstrated in this and other studies, the question occurred as to the relative influence of the stage of lactation and the environmental temperature on the percentage of butter fat.

To study these relationships the data were segregated by seasons of freshening and were coded for each group to show the trend in the percentage of butter fat with the advance in the lactation. These data are presented in figure 2. In this graph the ordinate expresses the percentage of butter fat and the abscissa the month of lactation. The center month of the season of freshening is taken as the first month on the graph in each case. In order to more clearly indicate the effect of temperature the winter season of December, January, February is shown in solid line in each case.

Graph II
Trend in the Percentage of Butter Fat
for Groups Freshening in the Respective Seasons



Group II shows that almost irrespective of the stage of lactation the high points in the percentage of butter fat occur in the winter period and the low points in the summer period. This is especially evident in the group freshening in the winter season. The percentage of fat drops rapidly the first months of the lactation, remains at a low level for the six months period from March to August inclusive, and then rises rapidly to the highest point in December as a result of the combined influence of decreasing temperature and the advanced stage of the lactation.

This graphic study indicates that the environmental temperature exerts a greater influence on the percentage of butter fat than does the stage of lactation. This observation is further emphasized by a comparison of the coefficients of correlation shown in table 4.

TABLE 4

Coefficients of correlation for the relationship between the environmental temperature and the percentage of butter fat in cow's milk, and between the stage of lactation and the percentage of butter fat in cow's milk

SEASON OF FRESHENING	ENVIRONMENTAL TEMPERATURE	STAGE OF LACTATION
Spring	−0 895 ±0 039	+0 397 ±0 171
Summer	−0 579 ±0 169	+0 298 ±0 178
Winter	−0 517 ±0 143	+0 429 ±0 159
Fall	−0 328 ±0 174	+0 074 ±0 194

It is noted from table 4 that in every case the coefficients for the correlation between the environmental temperature and the percentage of butter fat for the groups freshening in the respective seasons are greater than those for the relationship between the stage of lactation and the percentage of butter fat for the groups freshening in the respective seasons.

The similarity between the coefficients for the winter freshening group is explained by the fact that the relative influences of the stage of lactation and the environmental temperature are essentially the same throughout with the exception of the fifth to eighth months of lactation in which case the percentage of butter fat is noticeably depressed contrary to the normal trend at this stage of lactation due to the marked increase in environmental temperature in this period.

SUMMARY

1. This study confirms previous conclusions that there is a very close inverse correlation between the environmental temperature and the percentage of butter fat in cow's milk. This relationship may be expressed by the coefficient of correlation:

$$r = -0.872 \pm 0.046$$

2. This relationship assumes a distinctly linear regression and is characterized by the regression equation:

$$y = 4.41 - 0.0064\ X$$

3. The same relative relationship was found to maintain in each of the four dairy breeds studied, though marked by greater fluctuations in the higher testing breeds.

4. It was found that when the stage of lactation was eliminated the amount of milk produced by a group of cows in a given period did not appreciably affect the percentage of butter fat in that milk. The coefficient of correlation for this relationship between milk production and percentage of butter fat was:

$$r = -0.167 \pm 0.190$$

5. Environmental temperature exerts a greater influence on the percentage of butter fat of cow's milk than does the stage of lactation.

REFERENCES

(1) Eckles, C. H.: Jahreszeitliche Schwankungen Prozentischen Fettgehaltis in Kuhmilch. Milchwirtschaftliche Centralblatt, 1909, vol. 448.

(2) White, G. C., and Judkins, H. F.: Variations in the fat, solids not fat and total solids in cow's milk. Conn., Storrs Station, Bul. 94, 1918.

(3) Hills, J. L.: The effect of weather upon quality and quantity of milk. Vermont Agr. Exp. Sta. Rpt., 1892, 128.

(4) Brooks, N. P.: Experiment in warming a stable for dairy cows. Mass. Hatch Sta. Rpt., 1895, 39.

(5) Hays, W. P.: The effect of environmental temperature on the percentage of fat in cow's milk. Jour. Dairy Sci., 1926, ix, 219.

(6) Weaver, E., and Matthews, C. A.: The influence of temperatures and certain other factors upon the percentage of fat in milk. Iowa Agr. Exp. Sta. Research Bul. 107, 1928.

(7) Clothier, R. W.: Seasonal variations in the butter fat content of milk in Southern Arizona. Proc. Soc. Prom. Agr. Sci., 1919, xxxix, 75.

(8) Hooper, J. J.: Studies in dairy cattle. II. Milk production. Kentucky Agr. Exp. Sta. Bul. 248, 1923.

(9) Ragsdale, A. C., and Turner, C. W.: The seasonal variations of the per cent of fat in cow's milk. Jour. Dairy Sci., 1922, v, 544.

(10) Ragsdale, A. C., and Brody, S.: The effect of temperature on the percentage of fat in milk. A first report. Jour. Dairy Sci , 1922, v, 212.

(11) Ragsdale, A. C , and Turner, C. W.: The stage of lactation as a factor in the variation of the per cent of fat in cow's milk. Jour. Dairy Sci., v, 22.

A STUDY OF METHODS FOR DETERMINING NUMBERS OF MOULDS AND YEASTS IN BUTTER

2. THE INFLUENCE OF TEMPERATURE AND TIME OF INCUBATION*

A. H. WHITE AND E. G. HOOD

Division of Dairy Research, Dept. of Agriculture, Ottawa, Canada

In routine quantitative microbiological tests of a control nature, temperature and time of incubation are important factors in the procedure of methods, especially if results from different laboratories are to be comparable. Temperature affects not only the ability of microörganisms to grow but also the rate of growth and should be such as to provide optimum conditions of growth for the types of organisms to be determined. The time factor in routine work is also important from the standpoint of availability of results and economy in laboratory equipment, especially in commercial laboratories.

A review of the literature reveals that there is considerable variation in the temperatures and incubation periods employed by various workers in determining the number of moulds and yeasts in butter. Hood and White (1) recommended a temperature of 25°C. for 5 days and this procedure is being used with satisfactory results in Canadian laboratories. Lund (2) employed a temperature of 25°C. counting Oidia at three days and yeasts and Penicillium at 5 days. Hunziker (3) recommended room temperature for 5 days, and Macy (4) used room temperature (20° to 25°C.) making the counts at 3 days. Bouska and Brown (5) found that yeasts and Oidia were of sufficient size to count at two or three days at room temperature.

Abbott (6) and Stiritz (7) employed a temperature of 30°C. for 5 days with the former counting moulds at 48 hours. McKay (8) stated that in the laboratory of the American Association of Creamery Butter Manufacturers plates for mould and yeast

---

* Received for publication April 17, 1931.

counts were incubated at 36°C. for 48 hours and a magnifying glass used in counting. Brown (9), some time later, stated that the procedure used was to incubate plates for 72 hours at 75°F. (24°C.). Parfitt (10) reported that he found many of the commercial laboratories using 37°C. for 48 hours, and Clerkin (11) used a temperature of 20°C. for 5 days.

The work of several investigators also shows a wide variation in temperature requirements for different species of moulds and yeasts. Harrison (12), in a study of 70 yeast cultures isolated from Canadian cheese, found that only 20 grew as well or better at 37°C. than at 25°C., while 30 gave very scant or no growth at 37°C. Hammer and Cordes (13) found the optimum temperatures for lactose fermenting yeasts from dairy products to be 37°C. for one species and 30°C. for another. Nelson (14) reported that some of the "common white" yeasts grew well at both room temperature and 37°C., while others grew well at room temperature but poorly or not at all at 37°C. Guilliermond (15) stated that for several of the species of pink or red yeasts the optimum temperature for budding was 22° to 25°C., while at temperatures of 30°C. some became weakened and at 37° to 38°C. many of the yeasts stopped vegetating.

Thom (16) found that 56 species of Penicillium grew normally at 15° to 25°C., the temperature affecting the rate of growth only, but that the green forms were prevented from growing at 37°C. Thom and Church (17) in a study of Aspergilli found that their laboratory cultures grew freely between 20°C. and 33°C.

The wide divergence in the temperature and time of incubation employed in making mould and yeast counts of butter made it seem advisable to study the influence of these factors on mould and yeast counts of butter with a view to the adoption of a uniform temperature and incubation period in order that results from different laboratories would be comparable.

METHODS

This study was carried out in two parts, in the first part comparative mould and yeast counts were made on 39 samples of butter and in the second part 40 cultures of representative yeasts isolated from the butter plates were used.

The butter samples included all grades of pasteurized and unpasteurized Canadian butter, the first 24 being from commercial lots while samples 25 to 39 were from exhibition butter. A preliminary count was made on the first twelve samples and suitable dilutions were then plated. Samples 13 and 24 were plated in dilutions of 1 cc. and 0.01 cc., while the majority of the exhibition butters were plated in only one dilution after the count for mould and yeast score had been made. For 1:10 dilutions, 10 cc. of butter were used in a 90-cc. water blank and for 1:100 dilutions, 1 cc. of butter was used in 99 cc. of water.

The yeast cultures were first picked to malt agar slopes and after good visible growth were transferred to malt broth. The broth cultures were then plated in high dilutions on malt agar and a well isolated colony from each culture was then transferred to a malt agar slope on which the cultures were carried. Incubation temperature was 25°C. for all transfers.

Before using for comparative counts, the yeast cultures were inoculated into malt broth and after 18 to 24 hours incubation at 25°C. a loopful of the broth culture was transferred to a 99 cc. water blank and 1 cc. of this dilution was used for plating.

Duplicate plates of each dilution were prepared for incubation temperatures of 25°, 30° and 37°C. The plates were counted after 48 hours and again after 5 days.

The medium used was Bacto malt agar, dehydrated, and acidified to pH 3.5± with a 5 per cent lactic acid solution just before using.

Counting was done with the aid of a hand lens as recommended in Standard Methods of Milk Analysis (18) and the results expressed as the average of the duplicate plates.

RESULTS FROM PLATING BUTTER SAMPLES

In table 1 are given the yeast counts of the butter samples for the different temperatures and incubation periods studied.

The data presented show that for the majority of samples there was not a significant difference in the counts at 25° and 30°C. for incubation periods of 48 hours and 5 days, but that there was a decided decrease in the counts at 37°C. after both periods of in-

TABLE 1

Comparison of yeast counts from butter at different temperatures and incubation periods

SAMPLE NUMBER	YEASTS PER CUBIC CENTIMETER					
	25°C		30°C.		37°C	
	48 hours	5 days	48 hours	5 days	48 hours	5 days
1		2,200	1,650	2,200	14	43
2		2.555	2,565	2,550	40	200
3		526,000	583,000	561,000	12,000	11.500
4		355	260	300	3	4
5		2,600	1,050	1,400	29	31
6		9,850	8,700	9,600	14	68
7	435	455	460	440	250	285
8	6,450	6,450	4,150	4,350	2,050	2,300
9	5,915	5,880	2.725	3,455	505	780
10	1.065	1,110	835	970	415	410
11	1,375	1,530	770	810	35	380
12	34,600	34,700	32,900	32,650	20,800	20,350
13	21,700	25,300	19,900	21,550	2,150	2,400
14	1,250	1,400	1,200	1,450	265	266
15	73	132	71	124	37	95
16	5,750	7.750	4,850	6,000	85	250
17	9,800	12,800	6,200	8,800	50	179
18	3,400	4,600	2,450	3,300	700	800
19	13,400	15,050	8,000	13,800	65	110
20	132	187	139	197	13	22
21	87,900	95,300	69,350	85,200	2,300	2,350
22	43,150	50,550	6,100	34,300	544	784
23	5.000	8,200	4.600	5,650	133	133
24	13,050	13,600	12,250	12,500	1,100	1,300
25	32	55	29	75	9	25
26	1	3	1	2	1	3
27	15	24	13	26	12	23
28	45	65	117	117	139	117
29	94	126	91	124	13	18
30	54	61	59	78	6	17
31	102	119	60	90	18	36
32	600	930	380	640	0	0
33	200	230	250	260	100	120
34	300	340	310	350	0	0
35	10	40	20	20	30	30
36	20,100	21,500	19,200	20,300	1,300	1,300
37	5,350	6,030	1,970	5,260	130	130
38	250	420	360	480	70	100
39	950	1,400	1,040	1,240	0	5
Averages ..	*8,562	22,048	20,464	21,581	1.167	1,201

* For 33 samples only.

cubation. The results, however, show that at 5 days 28 samples had higher counts at 25° than at 30°C. although generally the increase was small. Even at 48 hours, 23 samples had higher counts at 25° than at 30°C. and in some cases the difference was quite noticeable, especially for sample 22. With the exception of sample 28, which was comparatively low count butter, all samples had lower counts at 37° than at 25° and 30°C., and in the majority of cases the differences were very marked, 3 samples failing to show any yeast colonies at the high temperature.

The average count of all cultures and counts of the majority of individual samples show a slight increase at all temperatures at 5 days as compared to 48 hours incubation. Generally the differences were of no practical significance but in a few cases at 30° and 37°C. the counts at 5 days were noticeably higher than at 48 hours. For 25°C. 31 samples had higher counts at 5 days than at 48 hours, for 30°C. 33 samples showed higher counts at 5 days, and for 37°C. the counts at 5 days were higher than at 48 hours for 29 samples.

In a few cases at each temperature the counts at 48 hours were higher than those recorded at 5 days which was probably due to two or more contiguous colonies growing together and counted as one at 5 days, while at 48 hours they may have shown as separate colonies under a hand lens.

The methods employed by various workers indicated that with the higher temperatures a shorter incubation period was used. When the yeast counts at 25°C. for 5 days are compared to the counts at 30° and 37°C. for 48 hours, it will be noted that the average counts at 25° and 30°C. for the respective incubation periods do not show a significant difference. This is largely due to the numerical difference in the count of sample 3 in favour of a temperature of 30°C. which is relatively much higher than the counts of the other samples. The average counts at 25°C. for 5 days and 30°C. for 48 hours, however, do not indicate the considerable differences in the counts for individual samples. The results show that 34 samples had higher counts at 25° than at 30°C., and in many cases the differences were quite marked. On a percentage basis the counts varied from 0.4 to 88 per cent and for 16 samples the counts at 30°C. for 48 hours were over 40 per cent lower than at 25°C. for 5 days. The counts at 37°C. for 48 hours are very much lower for most samples than at 25°C. for 5 days or at 30°C. for 48 hours.

TABLE 2

Comparison of mould counts at different temperatures and times of incubation

SAMPLE NUMBER	MOULDS PER CUBIC CENTIMETER					
	25°C		30°C.		37°C	
	48 hours	5 days	48 hours	5 days	48 hours	5 days
1	0.0	4.5	7.5	8.5	0.0	0.0
2	0.0	5.5	3.5	5.5	0.0	0.0
3	87,500.0	87,500.0	86,000.0	75,000.0	70.0	70.0
4	0.5	60.5	5.0	31.0	0.0	0.0
5	1.5	8.5	1.5	9.5	0.0	0.0
6	20.0	27.5	38.0	30.0	1.0	3.0
7	5.0	5.5	8.5	8.5	0.5	3.5
8	15.0	16.5	12.5	14.5	0.0	1.0
9	2.0	2.0	0.5	1.0	0.0	0.0
10	8.0	8.5	6.5	6.0	0.0	0.0
11	2.0	4.0	1.0	1.5	0.0	0.0
12	1,350.0	1,400.0	1,450.0	1,450.0	70.0	90.0
13	78.0	100.5	64.0	73.0	0.5	0.5
14	38.0	51.0	38.0	43.5	1.0	1.5
15	1.0	8.0	0.5	3.5	0.0	2.0
16	11.5	12.0	11.0	11.0	8.0	8.0
17	1.0	1.0	0.5	1.0	0.0	0.0
18	3.0	7.0	3.0	6.0	0.0	1.0
19	58.5	61.0	56.5	62.0	0.0	0.5
20	9.0	12.0	7.0	11.0	0.0	0.0
21	7.0	10.0	0.0	4.5	0.0	1.0
22	56.0	71.0	52.0	53.5	0.5	1.0
23	28.5	58.0	14.5	14.5	1.0	1.0
24	22.5	60.0	14.5	15.0	22.0	22.0
Averages....	3,717.0	3,729.0	3,658.0	3,202.0	7.3	8.6

In table 2 are shown the mould counts of 24 samples of commercial butter at different temperatures and incubation periods. The mould counts for the exhibition butters are not given as in most cases there were none or only a few colonies growing on the plates.

The data show that there was no marked variation in the mould counts for temperatures of 25° and 30°C. at 48 hours and 5 days incubation, but that the counts at 37°C. were very much lower in most cases than at 25° and 30°C., and for 9 samples moulds failed to grow at 37°C. when colonies were present on the plates incubated at the lower temperatures. The results of individual samples, however, show that 16 samples had higher counts at 25° than at 30°C., 2 samples had the same count and 6 samples had higher counts at 30° than at 25°C. for 5 days. The same general trend is found in the counts at 48 hours.

When the counts at 25°C. for 5 days are compared to those at 30° and 37°C. for 48 hours, it will be noted that both average and individual counts vary only slightly at temperatures of 25° and 30°C., while the counts at 37°C. are much lower.

The counts after the two different incubation periods show only slight variations for all three temperatures. At 25° and 37°C. the average counts are slightly higher at the 5 days incubation period, while the average count at 30°C. is higher at 48 hours due to the numerical difference in the mould counts of sample 3, which is very much higher than the counts for other samples. Excluding sample 3, the counts at 5 days are higher than for 48 hours at 30°C.

While the differences in the mould counts of individual samples at the different incubation periods for each temperature were of no practical significance, the results showed that for 25°C. 21 samples had higher counts at 5 days than at 48 hours, and for 30°C. 17 samples had higher counts after the longer incubation period. For a temperature of 37°C. there was practically no variation in the counts due to the small number of mould colonies appearing on the plates.

RESULTS FROM PLATING PURE YEAST CULTURES

In table 3 the colony counts for the yeast cultures at different temperatures and times of incubation are tabulated. No attempt was made to identify the yeasts other than to note the colony growth and colour on malt agar. For convenience, the cultures were grouped together in the table. The first 29 cul-

TABLE 3

Colony counts of yeast cultures at different temperatures and times of incubation

CULTURE NUMBER	25°C.		30°C.		37°C.	
	48 hours	5 days	48 hours	5 days	48 hours	5 days
1	466	483	479	487	455	463
2	491	513	448	454	0	0
3	525	535	573	570	498	507
4	480	486	464	470	469	469
5	322	339	329	338	106	123
7	358	374	378	389	389	396
8	537	551	528	528	484	489
9	169	171	145	171	0	0
10	89	92	65	68	9	10
13	823	836	796	814	40	123
14	365	368	294	363	0	0
15	155	168	124	155	0	0
16	474	497	461	496	0	0
17	321	341	316	333	0	0
19	120	121	135	135	139	145
20	179	181	186	189	160	163
24	235	247	216	250	0	0
25	279	285	287	291	0	0
26	163	200	35	132	0	0
27	354	369	322	339	0	0
28	259	260	242	248	0	0
30	619	638	629	650	593	602
31	177	193	190	203	154	178
34	79	81	90	93	83	87
35	209	215	202	213	0	0
37	181	184	173	184	117	165
38	107	109	100	100	91	95
39	524	537	482	490	0	0
40	307	345	325	341	132	238
6	253	260	246	251	0	0
12	295	296	269	279	0	0
18	86	86	91	91	0	0
21	260	267	273	285	0	0
29	280	309	309	325	0	0
32	220	245	204	210	0	0
33	127	135	123	129	0	0
36	219	239	234	252	0	0
22	59	63	0	5	0	0
23	132	194	35	99	0	0
41	37	41	0	10	0	0
Averages....	283	296	270	286	98	106

tures, from 1 to 40, were different types of white yeasts; cultures 6 to 36, 8 in all, belonged to the pink yeasts; and the last three, cultures 22, 23 and 41, appeared to be identical to the species Chromotorula ochracea described by Harrison (12).

A comparison of the counts at different temperatures shows that at 37°C. 24 cultures, or 60 per cent, failed to make any growth even at a 5 day incubation period, 4 cultures made some growth at 37°C., but the number of colonies was appreciably less than at 25° or 30°C., while the counts for 12 cultures were comparable to the results obtained at the lower temperatures. Of the 24 cultures that failed to make any growth at 37°C., 13 were white yeasts while all the cultures of pink yeasts and the other chromogenic type failed to grow. The four cultures that had counts considerably lower at 37° than at 25° and 30°C. belonged to the group of white yeasts. At 30°C. all but 5 of the cultures made as good growth as at 25°C. The three cultures of the Chromotorula ochracea type did not grow well at 30°C., and cultures 10 and 26 did not make as good growth at 30° as at 25°C. All cultures made good growth at 25°C.

The data in table 3 also show that with few exceptions there was no significant variation in the counts at 48 hours and at 5 days for all three temperatures. At 25°C. there was practically no difference in the counts at 48 hours and at 5 days. At 30°C. the counts for cultures 23 and 26 were appreciably higher at 5 days than at 48 hours, and for cultures 22 and 41 there were a few colonies visible at 5 days that did not show at 48 hours even with the aid of a hand lens. At 37°C. 13 cultures showed very slight differences in the counts at 48 hours and at 5 days, 3 cultures had noticeably higher counts at 5 days, while 24 cultures failed to make any growth even at 5 days.

At all temperatures, however, the average counts were slightly higher at 5 days than at 48 hours.

DISCUSSION OF RESULTS

The most noticeable effect of temperature on the mould and yeast counts of butter and on the growth of yeasts in pure culture was found at 37°C. At this temperature the counts of both moulds and yeasts were much lower than at 25° and 30°C. and in many cases the types of moulds and yeasts present in the butter failed to make any growth. At 30°C. for most of the samples the counts were comparable to those obtained at 25°C., but in a few cases there was a noticeable decrease in the counts of yeasts. At 25°C. all moulds and yeasts made good growth and the counts were generally higher at this temperature.

The marked decrease in the counts of the butter samples at 37°C. was undoubtedly due to the inhibiting effect of this temperature on the growth of some of the types of moulds and yeasts present in the butter. Although no detailed study was made to identify the types of moulds and yeasts appearing on the plates, a few observations were made regarding the occurrence of some of the more easily recognized types at the different temperatures of incubation. It was noted, for example, that there were numerous colonies of pink yeasts on the plates from sample 29 at 25° and 30°C. but none were present on the plates incubated at 37°C. The inhibiting effect of a temperature of 37°C. on pink yeasts was further substantiated in the results obtained when pure cultures of this type of yeasts were plated. In butter samples 4 and 6, greenish coloured moulds, probably of the Penicillium type, were present on the plates at 25° and 30°C. but were not found at 37°C., and for samples 3, 7, 10, 12, 13, 14, 19, 22, 23 and 24, the plates showed numerous mould colonies of the Oidium lactis type at 25° and 30°C. but at 37°C. there was a decided decrease in the number of colonies. These results are quite in accord with the results of other workers who observed that many of the moulds and yeasts were not able to grow at 37°C. or at best gave only a scant growth.

The data indicated clearly that a temperature of 37°C. was unsuitable for the incubation of plates in the routine analysis of butter for moulds and yeasts as the counts obtained at this temperature gave a wrong picture of the mould and yeast content of the majority of the butters analyzed. Reports of mould and yeast counts at 37°C. would be misleading to the buttermakers and creamery managers in interpreting the results in terms of the sanitary conditions surrounding the manufacture of the butter.

Even at 30°C. the yeast counts of some of the butters were so much lower than those obtained at 25°C. that they gave a better rating to the butter in this regard than was warranted.

Observations on the plates incubated at 37°C. also showed that many of the yeast colonies took on a brownish "burnt" tinge. In almost every case the medium was so dried out that it was cracked around the edges and through the center, which may have been an inhibiting factor to the growth of some of the moulds and yeasts.

Although the data presented indicate that the length of the incubation period made no significant difference to the mould and yeast counts of the butter samples and the colony counts of the yeast cultures, it should be remembered that a good hand lens was used in counting at 48 hours. For the majority of the butter samples and cultures of yeasts the counts would have been appreciably lower at 48 hours if the counting had been done with the naked eye. Many of the colonies of yeasts were so small that they were only observed after a very careful examination of the plates with the hand lens, and would have been entirely overlooked otherwise.

With the pure cultures of yeasts it was observed that a few of the white yeasts and all of the pink yeasts developed to sufficient size so as to be counted easily with the naked eye at 48 hours, but with many of the cultures, the colonies were too small to be visible without the aid of a hand lens. At 5 days incubation,however, all yeast colonies, both from butter samples and from the pure cultures, were easily seen.

The counts of the moulds for incubation periods of 48 hours and 5 days showed very little difference and all the moulds were fairly easily observed at 48 hours with the naked eye. When mould colonies are numerous on the plate it would be better to count at 48 or 72 hours, as at 5 days some of the more closely situated colonies may have grown together and would be difficult to distinguish as individual colonies.

It was also noted that the size of the mould and yeast colonies that grew at the three temperatures employed did not depend so much on the temperature as on the length of the incubation period. The higher temperatures of 30° and 37°C. did not induce quicker development of the colonies so that they were more easily observed after a shorter incubation period. There was no noticeable difference in the size of the colonies able to grow at the temperatures used, and the counts of the plates incubated at 25°C. for 48 hours were made as easily and quickly as were the counts of plates incubated at 30° and 37°C.

If control laboratories making numerous analyses of butter for moulds and yeasts desire to use a shorter incubation period in order to make results more quickly available and to economize in equipment, the data obtained in this study indicate that there is no advantage in using a higher temperature than 25°C. At 25°C. the counts generally are higher than at 30° or 37°C. and from the standpoint of size of colonies and ease of counting, the plates incubated at 25°C. gave just as satisfactory results.

If an incubation period of less than 5 days and especially of only 48 or 72 hours is used it is necessary to use a good hand lens in counting or the yeast counts will be much too low.

SUMMARY AND CONCLUSIONS

The data obtained in this study show that the average mould and yeast counts of all samples and the counts for the majority of individual samples were slightly higher at 25° than at 30°C. for both incubation periods of 48 hours and 5 days, although the differences could not be considered of practical significance. A few of the yeast cultures, however, did not grow as well at 30° as at 25°C. as indicated by the number of colonies. At 37°C. the mould and yeast counts in almost all cases were appreciably lower than at 25° and at 30°C., due to the fact that some of the types of moulds and yeasts did not grow at all or made only scant growth at this high temperature.

When a temperature of 25°C. for 5 days was compared to 30°C. for 48 hours, there was a considerable variation in the yeast counts of individual samples in favour of the lower temperature for the longer incubation period. Many of the counts at 30°C. for 48 hours were over 40 per cent lower than at 25°C. for 5 days. The counts at 37°C. for forty-eight hours were very much lower than at the other two combinations of temperature and incubation period.

The length of the incubation period did not affect the mould and yeast counts to any marked degree at any of the temperatures when a good hand lens was used in counting after 48 hours. The results show, however, that in general the counts were higher at an incubation period of 5 days than at 48 hours. If counting at the shorter incubation period had been done with the naked eye the counts would have been appreciably lower than recorded, as many of the yeast colonies were not apparent without the aid of a lens. At 5 days moulds and yeasts were easily counted with the naked eye at all temperatures used.

The size of the colonies and rate of growth of the moulds and yeasts growing at all temperatures was not influenced to any noticeable extent by temperatures of 30° and 37°C. as compared to 25°C. but was due rather to the length of the incubation period. It was evident that even for the short incubation period of 48 hours there was no advantage in using a higher temperature than 25°C. from the standpoint of size of colonies and rapidity of growth.

The data presented indicate that if mould and yeast counts of butter analyzed at different laboratories are to be comparable a uniform temperature and incubation period should be adopted, and 25°C. for 5 days gave the most satisfactory results in this study. If laboratory conditions necessitate a shorter incubation period than 5 days, counting should always be done with the aid of a good hand lens.

REFERENCES

(1) Hood, E. G., and White, A. H.: Studies on moulds and yeasts in creamery butter. Part II, Pamphlet No. 92 N.S., Dept. Agric., Canada.

(2) Lund, T. H.: Microörganisms in creamery butter. Scientific Agriculture, June, 1922, i, no. 10.

(3) Hunziker, O. F.: The Butter Industry. 2nd edition, 1927, p. 590.

(4) Macy, H.: Mould and yeast counts and their relation to the composition of butter. Jour. Dairy Sci., September, 1927, x, no. 5.

(5) Bouska, F. W., and Brown, J. C.: The significance of oidia and yeasts in pasteurized butter. Reprint from University of Wisconsin Studies in Science, no. 2.

(6) Abbott, F. H.: Standardization and improvement of California butter. Bulletin 443, College of Agr. Univ. of Cal., November, 1927.

(7) Stiritz, B. A.: Yeast and mould counts and their relation to pasteurization of cream for buttermaking purposes. Jour. Dairy Sci., 1922, v, no. 4.

(8) McKay, G. L.: By correspondence to E. G. Hood, May, 1927.

(9) Brown, J. C.: Weekly letter of the American Association Creamery Butter Manufacturers, October 15, 1930.

(10) Parfitt, E. A.: By correspondence to E. G. Hood.

(11) Clerkin, P.: Estimation of yeasts and moulds in butter. Reprint from the Jour. Dept. Agric., Dublin, xxviii, no. 2.

(12) Harrison, F. C.: Cheese Torulae. From the transactions of the Royal Society of Canada, third series, xxi, section V, 1927

(13) Hammer, B. W., and Cordes, W. A.: A study of lactose fermenting yeasts in "yeasty" cream. Research Bul. 61, Iowa Agr. Exp. Station, September, 1920.

(14) Nelson, J. A.: A study of the "common white" yeasts found in dairy products. Jour. Dairy Sci., September, 1928, xi, no. 5.

(15) Guilliermond, and Tanner: The Yeasts. 1920.

(16) Thom, Chas.: Cultural studies of species of penicillium. Bul. 118, Bureau of Animal Industry, U. S. D. A., February, 1910.

(17) Thom, Chas., and Church, Margaret B.: The Aspergilli. 1926.

(18) American Public Health Association. Standard Methods of Milk Analysis, 5th edition, 1928.

EFFECT OF SINGLE AND DOUBLE HOMOGENIZATION OF CREAM UPON COAGULATION BY HEAT AND RENNET AND UPON SEPARATION OF THE FAT*

B. H. WEBB

Research Laboratories, Bureau of Dairy Industry, United States Department of Agriculture, Washington, D. C.

The widespread use of the homogenizer in the processing of dairy products has made desirable a better knowledge of the physical changes which occur during homogenization. Alterations in the physical equilibria of the milk fat appear to be capable, under certain conditions, of changing the heat stability of the product fully as much as small shifts in pH or in salt balance. Consequently, a change in heat coagulation caused by a change in the distribution of the fat phase can be considered as a part of the general heat stability problem (17).

The effect of homogenization upon rennet coagulation is of interest because the results obtained indicate that changes in the condition of the fat phase may be of importance in rennet action. Workers in the past have not considered the effect upon rennet coagulation of an altered fat equilibrium such as is produced by homogenization.

In the manufacture of sterile cream two factors are at present causing some difficulty, the instability of the cream toward heat and the fat separation which occurs while in storage. A quantitative study has been made of the increase in heat stability which may result from rehomogenization and also of the degree of fat separation in homogenized cream during long periods of storage.

Burgwald (2) found the susceptibility of cream to feathering in coffee was increased when the product was homogenized.

The effect of single stage homogenization upon the heat stability of creams of different fat percentages when temperature

---

* Received for publication April 16, 1931.

and pressure are varied has been investigated by Webb and Holm (17). Maximum stability was always attained when a preheating temperature of 80°C. (176°F.) was used. Increases in homogenization pressure and in the fat content of the homogenized cream decreased stability. Further application of these principles to the manufacture of sterile cream has been shown (16).

The decreased stability of cream resulting from homogenization appears to be associated in some way with clumping of the fat globules (17). It has been noted (13) (5) (1) that fat clumping becomes greater as the pressure of homogenization is increased. Doan (7) (8) has shown that increased fat clumping is accompanied by increases in viscosity and in the tendency of cream to feather in coffee and by decreased stability toward coagulation by alcohol. The protein stability relations and changes in viscosity measured by these various methods agree with the data of Webb and Holm (17) obtained by measurements of the time of heat coagulation of homogenized creams.

During the last few years the two-stage valve has found much favor where homogenization of dairy products is practiced. Double homogenization has been shown by Doan (8) to affect protein stability in the same manner as when a two-stage valve is used. Doan also found clumping was decreased and stability increased at pressures of 3000–1000 pounds in comparison to the values obtained by a single homogenization at 3000 pounds.

Tracy and Ruehe (14) observed that the feathering of homogenized cream in coffee could be decreased by use of a second stage valve, providing the pressure used on the second homogenization did not exceed that used on the first valve.

In a recent study on ice cream mixes, Hening (10) has clearly shown that a very considerable reduction in the size of the fat clumps can be brought about in a mix homogenized at 2000 pounds pressure by rehomogenizing four times at 200 pounds pressure.

Either rehomogenization or use of a two-stage valve has been shown to decrease the viscosity of cream or ice cream mixes (12) (13) (9) (8) (10) (1). As has been mentioned, various results indicate that such a decrease in viscosity is associated with less clumping and greater heat stability.

Some evidence has been obtained showing that the concentration of calcium salts present in a homogenized product influences its heat stability. Tracy and Ruehe (14) showed that an excess of calcium present in homogenized cream may cause its feathering in coffee. Addition of calcium lactate to ice cream mixes before homogenization caused an increase in viscosity and size of fat clusters, according to the work of Hening and Dahlberg (11). Addition of these salts after homogenization did not produce this marked effect. Dahlberg and Marquardt (3) consider the cause of the formation of fat clusters in milk to be due to the presence of a maximum quantity of calcium ions, which, by imparting some positive charges to the weak, negatively charged fat globules, create an electrical affinity between the globules. The loss of creaming ability in heated milk was considered as being due to a precipitation of the calcium salts. This view has also been recently favored by Doan (6).

EXPERIMENTAL

Effect of homogenization upon coagulation by heat and by rennet

The homogenizer used was a single stage Progress machine of 60 gallons capacity per hour. In cases where double homogenization was used, the second pressure is written after the first but separated from it by a hyphen.

Since the homogenization of a cream so greatly affects its heat stability, it appeared that some interesting results might be obtained by making comparisons between heat coagulation and coagulation by other means. Accordingly, coagulation by heat, by alcohol, and by rennet were compared.

It has been shown by Doan (8) that homogenization of a cream affects its stability toward alcohol in the same manner as it influences its heat stability. These results were confirmed in this work and it was found that curves drawn for coagulation by alcohol would parallel those established for heat stability.

The stability of homogenized cream toward rennet coagulation was investigated. One-half gram of powdered rennet (Eimer and Amend, strength 1:30000) was dissolved in 200 cc. of distilled water. A solution was freshly made for each experiment.

The test was carried out as follows: 5 cc. of rennet solution was added to 230 cc. of cream at 37°C. (98.6°F.). After being mixed well in a 250-cc. bottle the cream was placed in a water bath at 37°C. (98.6°F.). It was slowly syphoned out of the bottle by means of a capillary tube and was allowed to run down the side of a clean glass beaker against a dark background so that the first trace of curd could be readily distinguished. The time of coagulation is given as the minutes which elapsed from the time the rennet was added until the first visible curd was produced. The limit of error of the method was found to be ±0.1 minute.

The method of heat coagulation used was the usual one employed in this laboratory. The product was placed in baby-size tins and sterilized at 120°C. (248°F.) in a pilot sterilizer. The sterilizer is so equipped that cans may be withdrawn at intervals without interrupting the experiment. The time of coagulation was taken upon the appearance of the first visible curd, the limit of error being ±1.0 minute.

Data obtained comparing coagulation by heat and by rennet are plotted in figure 1. Cream of 20 per cent fat was preheated to 60°C. (140°F.) and 80°C. (176°F.) but it was not held at the preheating temperature. It was homogenized immediately after heating and the coagulation time was then determined. This was repeated with cream containing 10 per cent fat. The unhomogenized check samples received the same heat treatment as the homogenized samples.

A study of figure 1 shows that homogenized cream coagulated by heat and by rennet gives the same type of curve. Increasing pressures of homogenization decrease stability. Low fat content and high preheating temperatures increase the stability of homogenized creams toward coagulation by heat and by rennet.

The effect of a variation in the milk-solids-not-fat content of creams containing 20 per cent fat upon their stability toward heat and rennet was investigated. The percentage of solids-not-fat was obtained by the addition either of water or of condensed skim milk as the case required. All samples were preheated to 80°C.

(176°F.) and half of each was then homogenized at 3000 pounds pressure. The time of coagulation by heat and by rennet was observed as described previously.

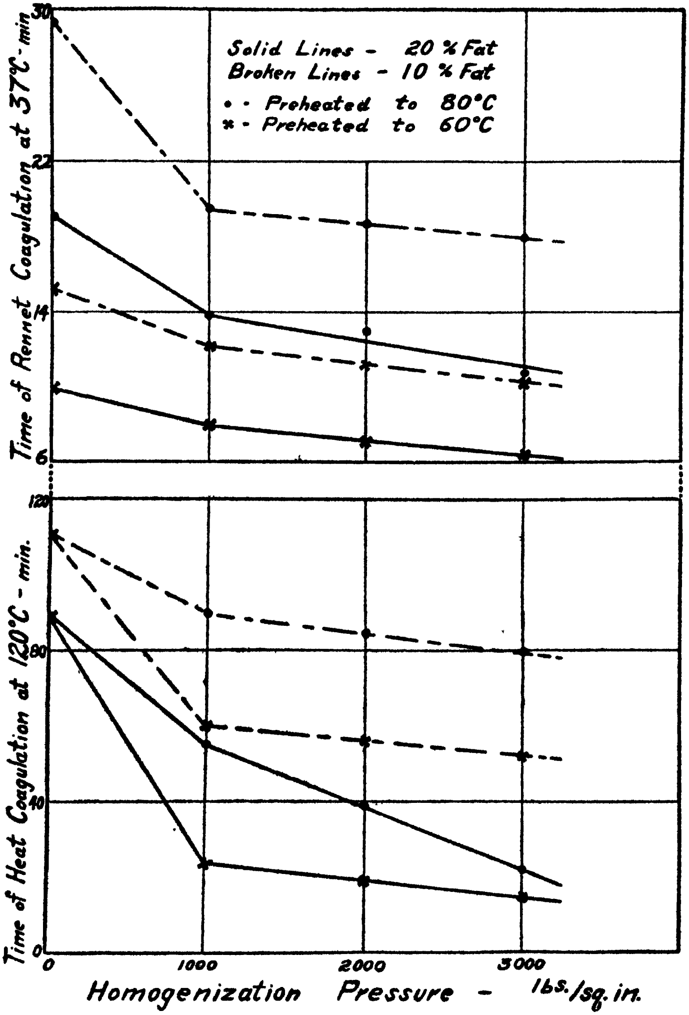


FIG. 1. SHOWING THE RELATIONSHIP OF HEAT COAGULATION AND RENNET COAGULATION TO THE PREHEATING TEMPERATURE AND HOMOGENIZATION PRESSURE OF CREAM OF 10 AND 20 PER CENT BUTTERFAT CONTENT

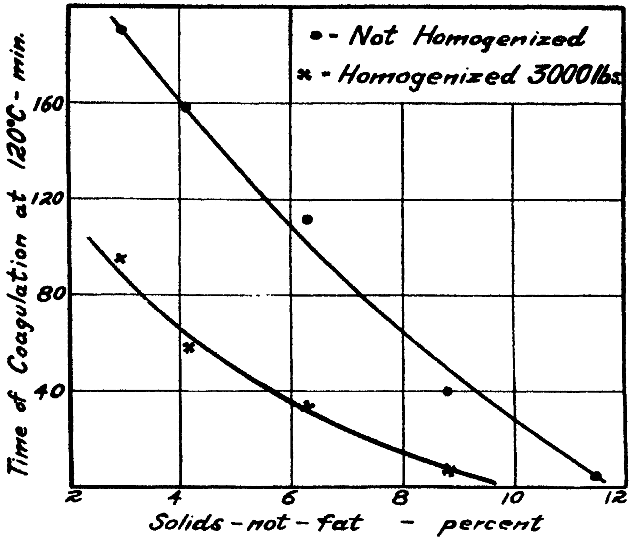


FIG. 2. SHOWING THE TIME OF HEAT COAGULATION OF CREAM OF 20 PER CENT FAT AS AFFECTED BY A VARIATION IN MILK-SOLIDS-NOT-FAT AND BY HOMOGENIZATION

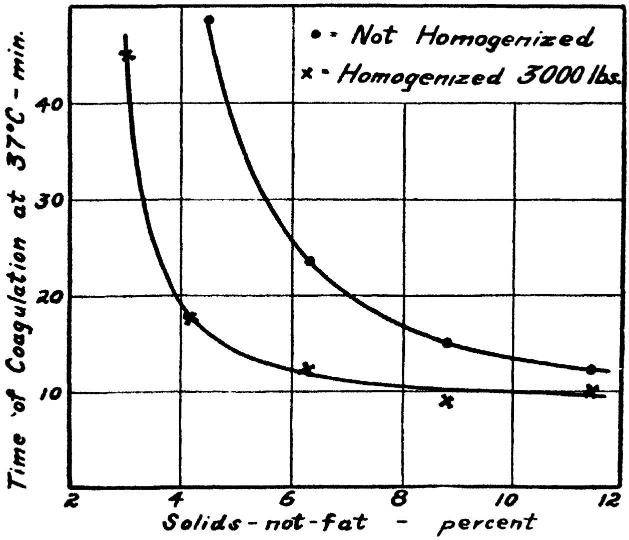


FIG. 3. SHOWING THE TIME OF RENNET COAGULATION OF CREAM OF 20 PER CENT FAT AS AFFECTED BY A VARIATION IN MILK-SOLIDS-NOT-FAT AND BY HOMOGENIZATION

In figures 2 and 3 are plotted the data obtained. Both homogenization of the cream and an increase in solids-not-fat are shown to decrease the time of coagulation by heat and by rennet.

Effect of double homogenization upon heat stability of cream

Creams varying in butterfat content from 15 to 30 per cent were homogenized twice at different pressures. All the samples were preheated to 80°C. (176°F.) before homogenization and in those cases where the cream was run through the machine a second time, it was reheated to 80°C. (176°F.) since the temperature dropped 5° to 10°C. before the second run could be made. The

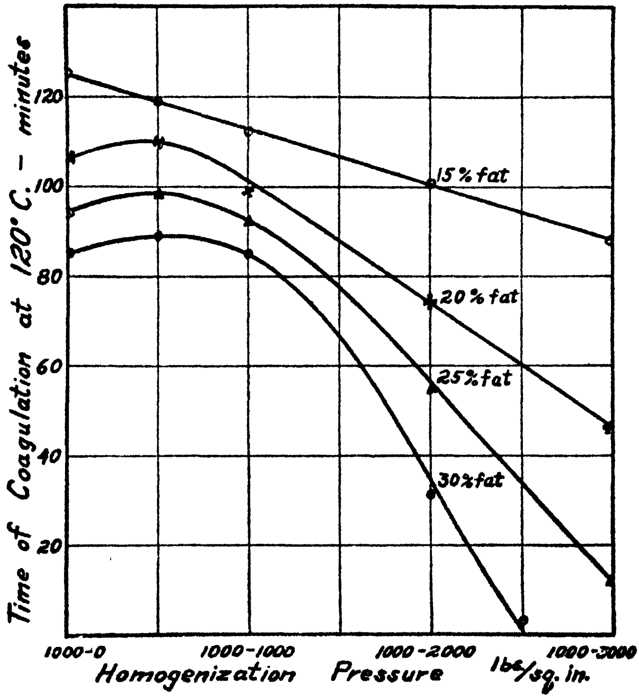


FIG. 4. SHOWING THE EFFECT OF DOUBLE HOMOGENIZATION UPON THE HEAT STABILITY OF CREAMS OF DIFFERENT BUTTERFAT CONTENTS

First homogenization pressures, 1000 pounds; second pressure varied heat stability of the samples was determined in the sterilizer at 120°C. (248°F.).

Representative data from this work, which was repeated several times, are plotted in figures 4, 5 and 6. Three initial pressures were used, 1000, 2000, and 3000 pounds. After treatment at these basic values, the cream was rehomogenized at various pressures.

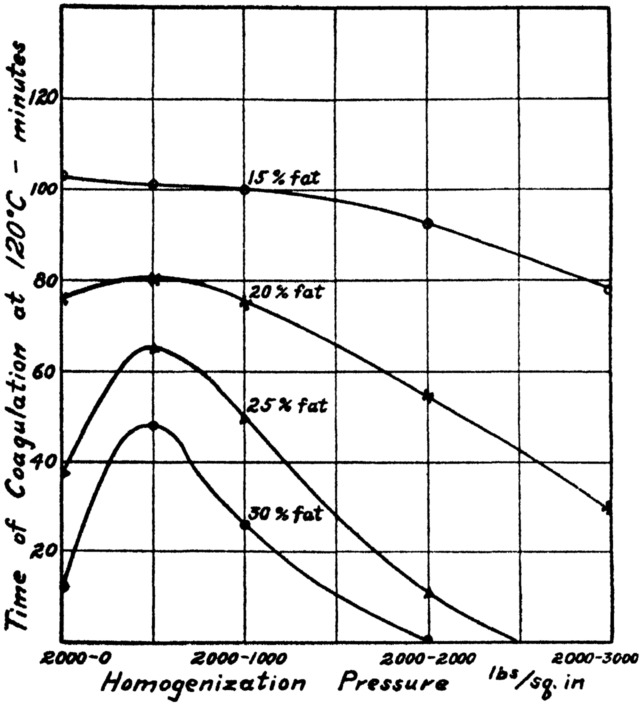


Fig. 5. Showing the Effect of Double Homogenization upon the Heat Stability of Creams of Different Butterfat Contents

First homogenization pressure, 2000 pounds; second pressure varied

The stability of cream of 15 per cent butterfat was in every case decreased by a second homogenization at pressures of from 500 to 3000 pounds. Cream containing 20, 25, and 30 per cent butterfat attained maximum stability when a second homogenization pressure of 500 pounds was used. With a fat content of 20 per cent, a second homogenization at 1000 pounds appeared of little or no advantage in increasing stability. In the case of creams containing 25 and 30 per cent butterfat, a marked improvement in stability results when a second homogenization of 500 to 1000 pounds is practiced. Double homogenization when the first stage was not over 3000 pounds and the second ranged from 2000 to 3000 pounds, always decreased stability over the single stage value.

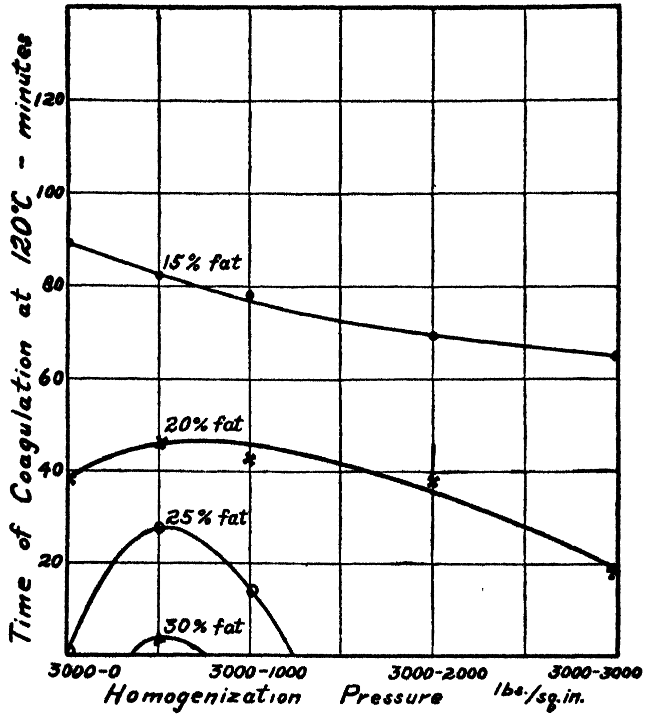


FIG. 6. SHOWING THE EFFECT OF DOUBLE HOMOGENIZATION UPON THE HEAT STABILITY OF CREAMS OF DIFFERENT BUTTERFAT CONTENTS

First homogenization pressure, 3000 pounds; second pressure varied

Rate of rise of fat in homogenized cream

In the preparation of sterile cream for the retail market, difficulty may be experienced in preventing excessive separation of the fat after periods of undisturbed storage.

Von Sobbe (19) noted a slight rise of fat from homogenized milk allowed to stand in tall glass cylinders. The milk originally contained 3.3 per cent fat. It gave in the upper third of the cylinder, after seventy-two hours, a test of 3.85 per cent fat when homogenized, and 14.50 per cent fat when not homogenized. Longer storage periods were not used.

The degree of fat separation in creams of 20.5 and 16.5 per cent fat homogenized at various pressures has been investigated after undisturbed storage of the samples at room temperature for long periods of time.

In this work the cream was preheated to 80°C. (176°F.), homogenized immediately at the required pressure, and sterilized in baby-size tins. The cans had a capacity of 180 cc. and the inside height of the can was 60.0 mm., this being the actual height of the cream column. The cans were placed in undisturbed storage at room temperature, which averaged about 25°C. (77°F.). After various intervals of standing the tins were opened without disturbing the fat layer and the contents were very carefully skimmed off in three equal layers—top, middle, and bottom. Analysis for percentage fat in each layer was made by the Babcock fat test.

Representative data are given in tables 1 and 2. Both the 20.5 and 16.5 per cent fat creams were homogenized once and some were rehomogenized at 500 to 4000 pounds pressure. In the cases where samples were homogenized twice, the second pressure was the same as the first. The sum of the fat percentages for the three layers of each sample should equal three times the percentage fat in the original cream. The deviation from this figure, 61.5 for 20.5 per cent cream and 49.5 for 16.5 per cent cream, represents the probable error in the method. This error varies in most cases within 0.5 per cent fat on each analysis.

The effect of single and double homogenization of 20.5 per cent

TABLE 1

Effect of homogenization and rehomogenization at various pressures upon the change in butterfat distribution during storage

Butterfat content of cream 20.5 per cent

HOMOGENIZATION PRESSURE	CREAM LAYER ANALYZED	FAT DISTRIBUTION AT END OF STORAGE PERIOD					
		2 days	1 week	2 weeks	4 weeks	7 weeks	16 weeks
pounds per square inch		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0	Top layer	53.0	59.0	61.0	63.0		
	Middle layer	9.0	1.0	1.6	0.4		
	Bottom layer	1.5	0.5	0.1	0.1		
500	Top layer	26.0	36.0	47.5	52.0	57.0	
	Middle layer	20.5	16.5	13.0	8.0	4.5	
	Bottom layer	16.5	7.5	3.2	0.4	0.2	
500–500	Top layer	23.5	30.0	39.5	48.0	52.0	56.0
	Middle layer	20.5	19.0	15.5	11.5	5.0	5.5
	Bottom layer	18.5	12.5	7.5	2.5	1.5	
1000	Top layer	23.5	28.5	35.0	42.5	51.0	
	Middle layer	21.0	19.5	17.0	13.0	9.0	
	Bottom layer	18.5	13.5	7.2	3.0	1.0	
1000–1000	Top layer	21.5	26.0	31.5	36.5		53.0
	Middle layer	21.0	20.0	19.5	16.5		10.0
	Bottom layer	20.0	16.5	12.5	8.0		0.0
2000	Top layer	22.0	24.0	27.5	33.5	39.0	49.0
	Middle layer	21.5	20.5	20.0	18.5	17.0	11.5
	Bottom layer	19.5	17.5	15.0	11.0	4.5	0.5
2000–2000	Top layer	22.0	22.5	24.5	28.0	33.0	45.0
	Middle layer	20.5	21.0	20.5	20.0	19.0	13.5
	Bottom layer	20.5	19.5	17.0	14.0	10.0	2.5
3000	Top layer	21.5	23.0	25.0	28.0	33.0	43.0
	Middle layer	21.0	20.5	19.5	20.0	19.0	17.5
	Bottom layer	20.5	18.0	16.5	13.0	10.0	0.6
3000–3000	Top layer	21.0	21.5	23.0	25.0	28.5	37.0
	Middle layer	21.0	21.0	21.0	21.0	20.0	18.5
	Bottom layer	21.0	20.0	18.0	15.5	13.5	6.0
4000	Top layer	22.0	24.5	25.0	28.0	30.5	
	Middle layer	20.5	22.0	22.5	24.5	25.0	
	Bottom layer	20.5	16.0	14.5	11.5	9.5	
4000–4000	Top layer					26.0	
	Middle layer					21.0	
	Bottom layer					15.5	

TABLE 2

Effect of homogenization and rehomogenization at various pressures upon the rate of butterfat separation during storage

Butterfat content of cream 16.5 per cent

HOMOGENIZATION PRESSURE	CREAM LAYER ANALYZED	TIME OF STORAGE AT ROOM TEMPERATURE					
		2 days	1 week	2 weeks	4 weeks	7 weeks	2½ years
pounds per square inch		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
0	Top layer	47.5	48.5	48.5			
	Middle layer	0.7	0.5	0.3			
	Bottom layer	0.1	0.1	0.1			
500	Top layer	20.5	25.0	29.0	36.0	41.0	44.0
	Middle layer	16.0	14.5	12.5	9.0	9.0	1.7
	Bottom layer	13.0	10.0	7.0	2.5		
500-500	Top layer	19.5	23.5	28.0	34.0	38.0	47.5
	Middle layer	16.0	16.0	13.5	10.0	10.0	2.7
	Bottom layer	14.0	11.0	7.0	3.5		
1000	Top layer	21.5	26.5	30.0	40.0	42.0	48.0
	Middle layer	16.5	14.5	11.5	8.0	8.0	1.8
	Bottom layer	12.0	8.5	5.5	2.3		
1000-1000	Top layer	19.0	22.5	26.0	31.5	39.5	46.0
	Middle layer	16.5	16.0	14.0	11.0	11.0	1.1
	Bottom layer	13.5	10.5	8.5	5.0		0.1
2000	Top layer	18.5	21.5	25.0	31.0	35.0	44.0
	Middle layer	16.5	16.0	14.0	12.5	14.0	2.4
	Bottom layer	14.5	12.0	9.5	6.0		
2000-2000	Top layer	17.5	20.0	21.5	25.0	30.5	42.0
	Middle layer	16.5	16.5	16.0	15.0	18.0	3.2
	Bottom layer	15.5	13.5	12.0	9.5		0.1
3000	Top layer	18.0	20.0	22.0	26.0	31.0	41.0
	Middle layer	16.5	16.5	16.0	13.5	20.0	3.3
	Bottom layer	15.0	13.0	12.0	8.5		
3000-3000	Top layer	17.0	18.5	19.0	21.5	26.5	39.5
	Middle layer	16.5	17.0	16.5	15.5	23.0	3.6
	Bottom layer	16.0	15.0	13.0	11.0		0.1
4000	Top layer	17.5	19.0	20.0	24.0	28.0	39.0
	Middle layer	16.5	16.0	16.0	14.5	20.0	4.3
	Bottom layer	15.5	14.0	13.5	10.0		
4000-4000	Top layer	17.0	17.5	19.0	20.0	23.0	37.0
	Middle layer	16.5	16.5	16.5	16.0	27.0	4.5
	Bottom layer	16.0	15.5	14.0	12.0		0.1

cream upon the rate of rise of the fat during storage is shown in figure 7. The figure is plotted from the values found in table 1. On the ordinate is the total fat content of the top layer of cream at the various pressures of homogenization, while against this is plotted the time of storage in weeks.

The data show that in general increasing pressures of homogenization retard fat separation somewhat in proportion to the pressure used. A second homogenization at the same pressure as the first further retards the rise of fat over that in cream which received the single stage treatment. Increasingly long periods of undisturbed storage cause increases in fat separation.

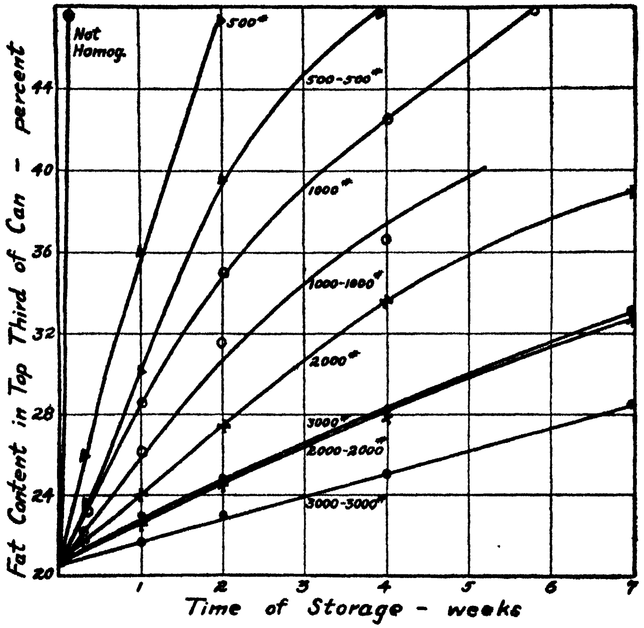


FIG. 7. SHOWING THE EFFECT OF SINGLE AND DOUBLE HOMOGENIZATION OF 20.5 PER CENT CREAM UPON THE RATE OF RISE OF THE FAT DURING STORAGE

The effect of rehomogenization upon the viscosity, heat stability, and rate of rise of the fat in a cream of 20.5 per cent butterfat is the effect reproduced in table 3. The values were all obtained upon the same cream and are therefore free from differences which often occur in various lots of cream. The homogenization temperature was 80°C. (176°F.) and the temperature of storage 23°C. (73.4°F.). The heat stability and relative viscosity data were obtained within five hours after homogenization.

TABLE 3

Effect of rehomogenization upon the viscosity, heat stability, and rate of rise of the fat on cream of 20.5 per cent butterfat

Temperature of homogenization, 80°C.

HOMOGENIZATION PRESSURE	RELATIVE VISCOSITY AT 20°C (WATER = 1)	TIME OF COAGULATION AT 120°C.	DISTRIBUTION OF FAT IN CAN AFTER TWO MONTH'S STORAGE		
			Top layer	Middle layer	Lower layer
pounds per square inch		*minutes*	*per cent*	*per cent*	*per cent*
0	3.75	168	62.5	0.9	0.2
500-0	3.80	117			
1000-0	4.20	106	51.5	7.0	0.6
1000-500	4.19	110	50.0	8.0	1.4
1000-1000	4.37	99			
1000-2000	4.82	75	40.5	16.0	6.5
1000-3000	5.55	46	33.0	18.5	8.4
2000-0	5.12	76	44.0	14.0	4.2
2000-500	4.75	81	43.0	15.0	5.5
2000-1000	4.87	75	39.5	15.5	7.5
2000-2000	5.12	55	35.0	15.5	9.0
2000-3000	5.85	30	31.5	19.5	11.0
3000-0	7.75	38	33.5	18.5	10.0
3000-500	5.50	47	34.5	19.5	9.5
3000-1000	5.70	43	33.5	19.0	9.5
3000-2000	5.80	38			
3000-3000	6.62	18	27.5	19.5	15.0

The data in table 3 summarizes the important stability changes which homogenization causes in cream. The close inverse relationship between viscosity and heat stability at various pressures stands out clearly. Rehomogenization at 500 pounds decreased viscosity and increased stability over single homogenization at

1000, 2000, and 3000 pounds. Increases above 500 pounds in the pressure used on the second stage increased the viscosity of the cream in the same manner as it decreased heat stability.

It is interesting to note that the separation of the fat decreases in the rehomogenized cream as the total pressure of both stages increases. A second pressure of 500 pounds makes little difference in fat separation, but rise of the fat is noticeably retarded when rehomogenization pressures of 1000 pounds or above are used.

Color and flavor of sterilized cream

In a previous report (16) the flavor of sterile cream observed after long periods of storage in glass containers was found much superior to the same product preserved in tins. Further observations have been made contrasting the flavor of 20 per cent sterile cream put up in glass, in plain tins and in two types of lacquered tins. After three months' storage, 25 of these samples were examined. Two cans were off flavor, but the remaining 20 did not appreciably differ in flavor from the 3 samples put up in glass. It would appear then, that sterile cream can be satisfactorily packaged either in glass, in lacquered or in plain tins, glass being perhaps the most suitable container.

A mixed group of 35 sterile cream samples which had been in storage at room temperature for from three to four and a half years were examined. Three were spoiled, while the others were still edible but had an old and disagreeable flavor.

The contrast in color between these creams when held in glass and when held in cans was interesting to note. The samples stored in glass darkened in color in a manner similar to the change noted during prolonged heating of the product. This change in color is a result of a change in the three color attributes, hue, brilliance, and chroma (18). The color observed in the creams held in tins had a greenish tinge, similar to that noted in cans of evaporated milk after prolonged storage. This change is due to an increase in chroma alone. The characteristic color of cream and evaporated milk stored in cans for long periods seems then to be dependent upon the presence of the tin.

DISCUSSION

From the work of several investigators (8) (14) (17), it is safe to assume that the clumping of the fat in homogenized cream is the cause of a lowered stability toward heat. Coagulation of such a hydrophilic colloid as casein depends upon the destruction of its water holding capacity and upon the flocculation of the denatured particles. The effect of numerous large clumps in cream may be of significance in the flocculation process. The size of the micelles and the velocity of their motion are factors which govern flocculation. Fat clumps in cream might easily provide suitable nuclei with an effect in proportion to their size, around which flocculation could proceed. On the basis of our present limited knowledge of the changes occurring during homogenization, the hypothesis that fat clumping is a major cause in lowering the stability of homogenized creams seems at this time to be correct.

It has been shown that homogenization affects the time of rennet and of heat coagulation in much the same manner. The lowered resistance of homogenized cream toward rennet coagulation may be due to any of the several changes which result from this treatment. The lowered pH (7), the increased size of the fat clumps, or the changed equilibrium arising from the heavy adsorption of protein onto the fat may affect the action of rennet on the cream.

The data plotted in figure 2 show the variation in heat stability with solids-not-fat in a 20 per cent cream, homogenized and unhomogenized. An increase in the concentration of milk-solids-not-fat greatly decreases the coagulation time of both the untreated and the homogenized creams. It will be noticed that as the solids content increases, the stability of the homogenized sample tends to approach the stability of the untreated product. This behavior is in accordance with the work of Doan (5) who showed that a critical ratio existed between serum solids and fat which controlled the degree of clumping during homogenization. In general, an increase in fat or a decrease in solids favored clumping. The low solids creams are clumped more as a result of homogenization than those of high solids content and their stability accordingly is lower in relation to the same unhomogenized creams than those samples containing a high percentage of serum solids. Hence the two curves tend to approach one another with increasing percentages of solids.

Optimum stability upon rehomogenization was found to be obtained when a pressure of 500 pounds was used. Use of a pressure lower than 500 pounds on the second stage was not investigated, although it is possible that stability might be further increased by such treatment. A second homogenization pressure of 1000 pounds increased the stability of high fat creams over the single stage treatment, but pressures of 2000 pounds or above always decreased the heat stability of the creams of each fat percentage used.

If the assumption is made that the degree of clumping in a cream is related to its heat stability, the data in figures 4, 5, and 6, would show the relative clumping tendency in rehomogenized creams. It appears probable that rehomogenization of cream of 20 to 30 per cent fat at pressures below 1000 pounds has a destructive effect upon fat clumps. In cream so treated the fat rises slower than the fat in cream homogenized only once. However, use of a rehomogenization pressure of 2000 to 3000 pounds decreases stability and might, therefore, be expected to increase the degree of fat clumping. But the fat in cream so treated also rises proportionally more slowly as the pressure of the second homogenization is increased.

It has been clearly demonstrated by Troy and Sharp (15) that the rate of gravity creaming of milk is chiefly dependent upon the fat forming into clusters. If rehomogenization of cream at high pressures further retards the rate of rise of the fat it can only be concluded on the basis of the work of Troy and Sharp that the individual fat aggregates have been decreased in size.

Babcock's (1) photomicrographs show that increases in homogenization pressure decrease the size but increase the number and density of the fat clumps. Rehomogenization appears to have a similar effect. A decrease in the total volume occupied by the clumps due to an increase in the initial pressure or to rehomogenization would account for the slower separation of the fat encountered under such conditions. Since the smaller-sized, tightly packed clumps outnumber the larger ones obtained at low pressures, more rapid heat coagulation should follow when high pressures of homogenization have been used.

Application of some of these results to the manufacture of sterile cream is of interest. The higher the pressure of homogenization which can be used without endangering the heat stability of the cream, the less fat will separate during storage. Under the conditions used in this work, preheating to 80°C. (176°F.) and homogenizing at 3000–500 pounds gave a sufficient margin of safety to allow proper sterilization in a cream of 20 per cent fat. The second homogenization at 500 pounds pressure is decidedly beneficial in increasing heat stability. Some fat separation will occur in this product but the fat layer is soft and readily disintegrates with a little shaking or stirring. The efficiency of homogenizers will vary as will also the properties of cream from different sources. The principles derived from the data reported here should be applicable under other operating conditions, but the actual stability figures will not be the same when different material and equipment are used.

SUMMARY

1. Homogenization of cream decreases its stability toward coagulation by heat and by rennet. Differences in homogenization temperature and pressure effect the time of coagulation by either heat or rennet in a similar manner.

2. Double homogenization at 80°C. (176°F.) when the second pressure is in the vicinity of 500 pounds per square inch is beneficial in increasing the heat stability of creams containing more than 15 per cent butterfat. Use of a pressure in excess of 2000 pounds during rehomogenization decreases the heat stability of a cream below that which is characterized by single stage treatment.

3. After several months' undisturbed storage noticeable butterfat separation occurs in sterilized sweet cream homogenized at pressures as high as 4000 pounds. Increasing pressures of homog-

enization retard the rate of rise of the fat approximately in proportion to the pressure used. The fat in cream which is rehomogenized separates to a lesser extent than the fat in cream which is homogenized only once.

REFERENCES

(1) Babcock, C. J. 1931 Some factors affecting the viscosity of cream. U. S. D. A. Tech. Bul. No. 249.

(2) Burgwald, L. H. 1923 Some factors which influence the feathering of cream in coffee. Jour. Agr. Res., xxvi, 541.

(3) Dahlberg, A. C., and Marquardt, J. C. 1929 The creaming of raw and pasteurized milk. N. Y. Agric Exp. Sta. (Geneva) Tech. Bul. 157.

(4) Doan, F. J. 1927 Viscolized milk and its detection. Jour. Dairy Sci., x, 501.

(5) Doan, F. J. 1929 Some factors affecting the fat clumping produced in milk and cream mixtures when homogenized. Jour. Dairy Sci., xii, 211.

(6) Doan, F. J. 1930 Studies on cream line formation. Pa. Agr. Expt. Sta. 43 An. Rept. Bul. 258, p. 27.

(7) Doan, F. J. 1930 Protein stability affected by homogenization of mixes. Ice Cream Review, xiii, no. 8, 40.

(8) Doan, F. J. 1930 The causes and prevention of cream feathering. Amer. Creamery and Poultry Produce Rev., lxxi, no. 9, 374.

(9) Doan, F. J., and Minster, C. H. 1930 The effect of the homogenization process on fat dispersion and casein stability of milk and cream Pa. Agr. Exp. Sta. 43 An. Rept. Bul. 258, p. 28.

(10) Hening, J. C. 1931 Some observations on the basic viscosity of ice cream mixes. Jour. Dairy Sci., xiv, 84.

(11) Hening, J. C., and Dahlberg, A. C. 1929 Effects of certain salts on the physical properties of ice cream mixes. Jour. Dairy Sci., xii, 129.

(12) Martin, W. H., and Dahle, C. D. 1925 The use of the two-stage valve. 38th An. Rept. Pa. Agr. Exp. Sta., p. 23.

(13) Reid, W. H. E., and Skinner, G. R. 1929 The effect of homogenization at different pressures on the physical properties of an ice cream mixture and the resulting ice cream. Mo. Sta. Res. Bul. 127.

(14) Tracy, P. H., and Ruehe, H. A. 1929 Factors affecting the heat coagulation of homogenized coffee cream. Univ. of Ill. Agr. Exp. Sta. Bul. 352.

(15) Troy, H. C., and Sharp, P. F. 1928 Physical factors influencing the formation and fat content of gravity cream. Jour. Dairy Sci., xi, 189.

(16) Webb, B. H. 1930 The sterilization of sweet cream for market purposes. Jour. Dairy Sci., xiii, 159.

(17) Webb, B. H., and Holm, G. E. 1928 The heat stability and feathering of sweet cream as affected by different homogenization pressures and different temperatures of forewarming. Jour. Dairy Sci., xi, 243.

(18) Webb, B. H., and Holm, G. E. 1930 The color of evaporated milks. Jour. Dairy Sci., xiii, 25.

(19) Von Sobbe, O. 1914 Die Bestimmung des homogenisations grades der milch. Milchwirtschaft Zentr., xliii, 503.

THE RELATION OF FEATHERING AND HEAT STABILITY OF CREAM TO FAT CLUMPING PRODUCED BY HOMOGENIZATION*

F. J. DOAN

Department of Dairy Manufactures, Pennsylvania State College, State College, Pennsylvania

The work reported in this paper deals with an investigation undertaken in an effort to determine whether there is a relationship between the degree of fat clumping in homogenized cream and the stability of such cream toward heat. This stability is of considerable importance to the milk dealer selling homogenized table cream and to the manufacturer of sterilized cream. Cream having poor stability toward heat will "feather" or curdle when added to coffee or when coffee is added to it. This condition is extremely objectionable and often leads to considerable difficulty with customers. In the production of sterilized cream, a high degree of stability is essential, otherwise it is practically impossible to use sufficient heat to render the product sterile without producing a graininess or coagulation. Webb (1) has recently shown that a minimum of 2500 pounds homogenizing pressure is necessary to prevent objectionable cream separation in this product and even under these conditions a slight, soft, cream layer is often present after a few months of storage. Pressures as high as 2500 pounds usually render even the best of cream comparatively unstable.

The "feathering" of homogenized table cream and the coagulation of sterilized cream are both regarded as a heat coagulation of the casein similar to that often encountered in the processing of evaporated milk. That there is a difference, however, in the

---

* Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station as Technical Paper No. 522. Preliminary report on this subject was made before the manufacturing section of the American Dairy Science Association at the annual meeting, Ames, Iowa, June, 1930. Received for publication April 17, 1931.

factors influencing heat coagulation in homogenized cream and in evaporated milk is illustrated by the fact that homogenization of cream greatly lowers the heat coagulation point (1) whereas homogenization of evaporated milk, even at high pressures, does not very materially affect the stability (2) (3).

Webb and Holm (4) have shown that the stability of cream toward heat is greatly altered by the degree of preheating and by the pressure of homogenization and have indicated a very definite reciprocal relationship between stability and viscosity for a given cream when heat treatment and homogenizing pressure are varied.

Tracy and Ruehe (5), in studying a case of cream "feathering," found that adjustment of the "salt balance" after the method suggested by Sommer and Hart (6) for evaporated milk, materially improved the resistance of homogenized cream toward "feathering" in coffee. The salt used to increase stability was sodium citrate but it was indicated that di-sodium phosphate and sodium bicarbonate would also act in like fashion.

In a recent report (7) these authors conclude that, "any treatment of the cream that causes the fat to clump or causes an increase in viscosity, tends to increase feathering." They state however that fat clustering is not the sole explanation since whipped cream which contains clusters, feathers to a lesser extent than the cream prior to whipping. The last statement, of course, is open to the criticism that the type of fat aggregates formed by whipping cream is entierly dissimilar to that obtained from homogenization.

After considering the experimental work mentioned, together with that of several others (8) (9) (10) (11) (12) (13), one interesting fact stands out. Practically all the methods, used or suggested for improving the stability of homogenized cream, decrease the tendency toward fat-clumping. It was therefore felt that the fat clumps themselves might play some positive rôle in inducing "feathering" and coagulation. To summarize briefly, the evidence in the literature on which this idea is based, the following facts relative to the stability of homogenized cream and the fat clumping therein, can be listed:

1. Increasing homogenizing pressures destabilizes cream toward heat. Such treatment likewise increases the degree of fat clumping.
2. Preheating cream at low temperatures [145°F. (62.8°C.) for thirty minutes or flash up to 170°F. (76.6°C.)], destabilizes the product toward heat, compared with higher heats or longer exposure. Such preheating will favor a higher degree of fat clumping than the more severe heat treatments.
3. Additions of soluble calcium salts or the use of hard water in coffee-making, increase feathering. Soluble calcium also promotes fat clumping when added before homogenizing.
4. Increases in the viscosity of cream are attributed generally to an increase in the degree of fat clumping and stability becomes less as viscosity increases.
5. Heating cream or milk after homogenization, and after the fat clumps have formed, does not materially change the degree of fat clumping (unless agitation is drastic) nor does it affect the stability nearly as much as heating prior to homogenization.
6. Additions of sodium citrate to cream prior to homogenization stabilize the product toward feathering in coffee and lessens the extent of fat clumping.
7. Increasing pressures of homogenization affect the fat clumping of evaporated milk practically not at all, and the stability but slightly, the reason for this being that the plasma-solids/-fat ratio is great enough to prevent noticeable clumping at any pressure.

EXPERIMENTAL

If there is a relationship between the degree of fat clumping and the stability of homogenized cream, any method of destroying the fat clumps or preventing their formation should result in a product which is more stable.

Mechanical destruction of fat clumps

The best argument for a belief that the fat clumps themselves affect stability was found in the results of some experiments where the clumps were destroyed mechanically after they had once formed in the cream, the stability of such cream being found improved thereby. The clumps were destroyed by: first, violent agitation; second, double homogenization; and third, the use of the two-stage valve on the homogenizer.

The stability of the samples was determined in three ways; by coagulation with alcohol, by feathering tests in boiling hot coffee, and by sterilizing trials with sealed tins at temperatures between 235°F. (112.8°C) and 242°F. (116.6°C.), the duration of heating being fifteen minutes.

The alcohol coagulation test was carried out by measuring 5-ml. portions of the sample into test tubes and determining the least amount of alcohol sufficient to produce flocculation of the protein. The dilution effect was controlled by using a sufficient amount of water in all tests to make the total addition of alcohol plus water equal to 10 ml. This test has been found to correlate fairly accurately with other heat stability tests.

Feathering tests in coffee were performed by adding 3 drops of the sample of cream to 10 ml. of coffee (prepared from powdered soluble coffee) in a test tube, immediately after the coffee had been brought to a boil.

Sterilizing trials were made by sealing several portions of a sample in tin cans and sterilizing in a pilot sterilizer at a definite temperature for fifteen minutes. Several temperatures were used in each experiment until a trial produced a series of samples which properly indicated the difference in stability. In other words, if all the samples in the first trial were coagulated, the temperature was reduced in the next trial; or if all were smooth and uncoagulated in the first trial, the temperature was increased until a trial produced a series of cans which properly indicated the comparative resistance of the samples to coagulation.

Table 1 shows the results of one typical experiment out of many that were performed. Portion (a) of the table shows the effect of agitation, of a vigorous type, in destroying the fat clumps and increasing the resistance of homogenized cream to coagulation. The samples are comparable with each other but not with those in portions (b) or (c) since each portion represents a different original lot of cream.

The degree of fat clumping is rated descriptively and also numerically, a rating of 6 being about as much clumping as it is possible to obtain with 20 per cent cream under any circumstance.

TABLE 1

Effect of destruction of fat clumps on the stability of homogenized 20 per cent cream, forewarmed and homogenized at 150°F. (65.5°C.) for thirty minutes

TREATMENT OF SAMPLE	DEGREE OF FAT CLUMPING	ALCOHOL NUMBER	VISCOSITY AFTER HOMOGENIZING	DEGREE OF FEATHERING IN COFFEE	DEGREE COAGULATED AFTER STERILIZING
a. Vigorous agitation					
					STERILIZED (235°F.) (110°C.)
1. Unprocessed	None (0)	6.8	38	–	– (Thin)
2. Homogenized 1000 pounds	Evident (1)	5.0	192	+	– (Medium)
3 Homogenized 3000 pounds	Very prominent (4)	4.0	625	++++	+
4. Lot 2 vigorously agitated	Evident (1)	5.2	103	±	– (Medium)
5 Lot 3 vigorously agitated	Prominent (3)	4.0	411	+++	– (Very heavy)
b. Double homogenization					
					STERILIZED (242°F.) (116.6°C.)
1. Unprocessed	None (0)	7.6	24	–	– (Thin)
2. Homogenized 1000 pounds	Very evident (2)	6.0	213	–	+
3. Homogenized 3000 pounds	Pronounced (5)	5.2	724	++++	++++
4. Lot 2 re-run at 1000 pounds	Not evident (0)	6.8	86	–	– (Medium)
5. Lot 3 re-run at 1000 pounds	Barely evident (1)	6.6	109	±	– (Heavy)
c. Two-stage valve					
					STERILIZED (239°F.) (115°C.)
1. Unprocessed	None (0)	7.2	33	–	– (Thin)
2. Homogenized 1000 pounds	Evident (1)	6.4	174	+	+
3. Homogenized 3000 pounds	Very prominent (4)	5.6	846	++++	++++
4. Homogenized 1000–500 pounds	Not evident (0)	7.0	67	–	– (Medium)
5. 3000–1000 pounds	Evident (1)	6.6	127	+	± (Very heavy)

The vicosity ratings are relative only and were obtained using a MacMichael Viscosimeter with a No. 32 wire at 60°F. (15.5°C.).

The unprocessed lots of cream were preheated at 150°F. (65.5°C.) for thirty minutes and then divided into five portions and treated as indicated. The samples described as vigorously agitated were stirred for a definite length of time with a high speed device which excluded air. All lots were cooled immediately in ice water and tests made within four hours.

It will be noted that in all cases destruction of the clumped formation of the fat resulted in an increased stability. Where the clumps were totally destroyed, the samples practically regained the resistance to coagulation which they exhibited before they were homogenized. This appears to be good evidence that the fat clumps themselves are mainly responsible for the loss of stability caused by homogenizing cream.

Increasing solids

In a previous study (9), it was found that fat clumping could be diminished by increasing the proportion of plasma solids in cream prior to processing. Thinking that an addition of solids, in the form of plain condensed skimmilk or skimmilk powder, to cream prior to homogenization, might increase its stability toward heat, a series of studies was made on this point. The evidence is not as clear cut as might be desired but it is quite definitely shown by the study reported in Table 2 that where samples of cream are greatly destabilized due to homogenization, extra solids present, if not in excess, prevent excessive clumping and do tend to stabilize the product. The opposite effect is also indicated, namely, that a lowering of the plasma solids concentration increases the degree of fat clumping and causes a loss of stability. In the case of added solids, it seems that stability is improved only in cases where homogenization has destabilized to such an extent, that the reduction of clumping (accomplished through the addition of solids) has a more beneficial effect than the solids themselves have a detrimental effect. The beneficial effect of added solids was not found to be nearly so effective as dual homogenization and where this method of homogenization is used, added solids always cause a loss of stability in fresh cream.

In the experiment illustrated by table 2, skimmilk powder was used as a source of added solids. Microscopic examination of the

TABLE 2

Effect of added solids in preventing fat clumping on the stability of 20 per cent cream forewarmed and homogenized at 150°F. (65.5°C.) for thirty minutes

TREATMENT OF SAMPLE	DEGREE OF FAT CLUMPING	ALCOHOL NUMBER	VISCOSITY AFTER HOMOGENIZING	DEGREE OF FEATHERING IN COFFEE	DEGREE COAGULATED AFTER STERILIZING (235°F) (110°C)
a. Normal plasma solids					
1. Homogenized 1000 pounds	Evident (1)	5.6	198	+	+
2. Homogenized 3000 pounds	Very prominent (4)	4.8	575	++++	++++
3. Homogenized 3000–1000 pounds	Barely evident (1)	5.8	56	−	− (Medium)
b. Plasma solids raised to 9 per cent (same basic cream)					
4. Homogenized 1000 pounds	Not evident (0)	6.2	183	−	− (Heavy)
5. Homogenized 3000 pounds	Evident (1)	6.0	528	+++	++
6. Homogenized 3000–1000 pounds	Not evident (0)	6.6	62	±	− (Medium)
c. Plasma solids raised to 11 per cent (same basic cream)					
7. Homogenized 1000 pounds	Not evident (0)	5.4	434	+++	++++
8. Homogenized 3000 pounds	Barely evident (1)	5.0	1,022	++++	++++
9. Homogenized 3000–1000 pounds	Not evident (0)	5.2	122	++	+++
d. Plasma solids lowered to 4.5 per cent (same basic cream)					
10. Homogenized 1000 pounds	Prominent (3)	5.2	178	++	+
11. Homogenized 3000 pounds	Very prominent (4)	4.8	321	++++	+++
12. Homogenized 3000–1000 pounds	Prominent (3)	5.4	206	+	+

samples revealed the effectiveness of the extra solids in reducing clumping and the withdrawing of solids in increasing clumping. It will be noted that stability values obtained by the three methods do not correlate exactly but in a large number of trials the variations were not great nor considered important. A critical examination of the table also shows clearly that there are two opposing factors operating in this experiment, one being the beneficial influence of a decrease in clumping; the other a detrimental influence due to an increasing amount of plasma solids.

TABLE 3

Effect of temperature of preheating and temperature of homogenizing on the degree of fat clumping and the stability of 20 per cent cream

SAMPLE HOMOGENIZED AT	DEGREE OF FAT-CLUMPING	ALCOHOL NUMBER	DEGREE OF FEATHERING IN COFFEE	DEGREE COAGULATED AFTER STERILIZING (240°F.) (115 5°C)
a. Pasteurized 145°F. (62.8°C.) for thirty minutes				
145°F. (62.8°C.)	Very evident (2)	7.4	+	+
100°F. (37 7°C.)	Pronounced (5)	7 0	+	+
80°F. (26 6°C.)	Very pronounced (6)	6.8	++++	++
b. Pasteurized 160°F. (71.1°C) for thirty minutes (same basic cream)				
160°F. (71.1°C.)	Evident (1)	7.6	–	– (Medium)
120°F. (48.8°C.)	Prominent (3)	7 6	–	– (Heavy)
80°F. (26.6°C.)	Very prominent (4)	7.4	+	++
c. Pasteurized 180°F. (82.2°C.) for five minutes (same basic cream)				
180°F. (82.2°C.)	Very evident (2)	7 2	±	±
130°F. (53 3°C.)	Prominent (4)	7.2	+	+
80°F. (26 6°C.)	Very pronounced (6)	6.6	++++	+++

Temperature effects

Much importance has been attached to the temperature of pasteurizing or preheating of cream from the standpoint of preventing an excessive loss of stability upon homogenizing (3). While it is a fact that the heat treatment prior to processing is very important, results obtained in these experiments indicate that the temperature of homogenizing is not to be overlooked.

Where cream is preheated at high temperatures and homogenized at low temperatures, the stability is only a little better than if preheated to a low temperature. Table 3 illustrates typical results obtained when cream is homogenized at various temperatures after being preheated at 145°F. (62.6°C.) for thirty minutes, 160°F. (71.1°C.) for thirty minutes and 180°F. (82.2°C.) for five minutes. It will be noted that the data are in general agreement with the findings of Webb and Holm (3) in that stability is at a maximum under a preheating treatment of about 160°F. (71.1°C.) for thirty minutes and falls when the degree of heat used increases over this amount. If the increased stability of preheated homogenized cream depended primarily on a precipitation of calcium and albumin, the higher heats should stabilize even more effectively than the 160°F. (71.1°C.) treatment (as in evaporated milk they do) but this is not found to be the case with homogenized cream. Stability parallels more nearly the degree of fat clumping than the temperature of preheating. If the heat effect alone, were the factor, homogenizing at high or low temperature after the preheating should make little difference in stability whereas actually, lowering the temperature prior to homogenization increases fat clumping and decreases stability.

Salt stabilizers

Previous investigators (5) have shown that buffer salts such as sodium citrate, di-sodium phosphate, sodium bicarbonate, etc., will render homogenized cream more stable and have attributed this effect to a change in the so-called "salt balance" whereby the normal excess of calcium is compensated by citrate, acid phosphate or acid carbonate ions. Experiments, carried out on homogenized cream using stabilizers seem to indicate that the beneficial effects may be due to the fact that these stabilizers tend to restrict clumping of the fat as well as to their ability to change the "salt balance." Some of the benefit may also be due to the action of these salts in reducing the hydrogen ion concentration of the cream. In any case, where the stabilizer is added after homogenization, the increase in stability is not nearly so marked as where the salts are added prior to homogenizing. Table

4 shows the data obtained in a trial using sodium citrate. Essentially the same results were obtained with other so-called salt stabilizers. It will be noted that two-stage homogenization had considerably more effect in increasing the stability of the cream homogenized at 3000 pounds than did the addition of the citrate after homogenizing. The degree of fat clumping is not changed apparently by citrate addition where such addition is made after homogenization and the stability is not affected greatly. Where citrate was added prior to homogenizing the stability was greatly improved but the degree of fat clumping was at the same time considerably diminished as evidenced by microscopic examination and by viscosity readings.

TABLE 4

Effect of sodium citrate added at different stages of processing on the fat clumping and the stability of 20 per cent cream, preheated and homogenized at 150°F. (65.5°C.) for thirty minutes

TREATMENT OF SAMPLE	DEGREE OF FAT CLUMPING	ALCOHOL NUMBER	VISCOSITY AFTER HOMOGENIZING	DEGREE OF FEATHERING IN COFFEE	DEGREE COAGULATED AFTER STERILIZING (240°F) (115.5°C)
Normal cream					
1. Homogenized 3000 pounds . . .	Prominent (3)	5 0	430	+++	+++
2. Homogenized 3000–1000 pounds.	Not evident (0)	5.6	81	–	– (Medium)
0.10 per cent sodium citrate added after homogenizing (same basic cream)					
3. Homogenized 3000 pounds.	Prominent (3)	5 6	392	++	+
4. Homogenized 3000–1000 pounds.	Not evident (0)	6.0	74	–	– (Thin)
0.10 per cent sodium citrate added before homogenizing (same basic cream)					
5. Homogenized 3000 pounds.	Barely evident (1)	7.2	218	–	– (Thin)
6. Homogenized 3000–1000 pounds	Not evident (0)	7.4	60	–	– (Very thin)

SUMMARY AND CONCLUSIONS

The experimental work described indicates that the degree of fat clumping in normal, sweet, homogenized cream is probably the most important single factor affecting the stability of such cream toward heat. The fact that mechanical destruction of the fat clumps improves stability would seem to preclude the possibility that both fat-clumping and stability are influenced by a third factor and to show that the relationship is one of direct cause and effect. Other evidence in the literature of the subject also tends to confirm this opinion but not so unanswerably as the fact that the cream is comparatively unstable when clumps are present and stable when they are destroyed.

As to the manner in which the fat clumps lower the heat coagulation point of cream, a physical property heretofore ascribed entirely to the condition of the casein and the properties of the serum, it is not felt that enough evidence is at hand to formulate a theory. However it is believed that the fat clumps act as a structure on which the casein coagulates more readily than it would otherwise. Perhaps the action is accelerated by the fact that the casein adsorbed on the fat globules, having lost its mobility and according to some concepts of adsorption, its hydration, is thereby rendered more susceptible to agents of coagulation. If this is true, then fat clumps bring a large proportion of such easily precipitated casein into intimate contact and presumably would favor coagulation. Naturally the subject is a complicated one and it is very difficult to reconcile all the demonstrable facts with any theory. As a result of this study, however, a few definite statements can be made relative to the production of sterile cream and homogenized table cream of high stability.

Double homogenization or the use of a two-stage valve is very effective in improving the stability of cream compared with a single processing treatment and constitutes a better method, perhaps, than the addition of salt stabilizers although in some cases both treatments may be necessary. Dual homogenization makes it possible to use a much higher total pressure and thus to obtain better homogenizing efficiency, which is important, particularly in the manufacture of sterile cream. Naturally, dual or two-stage homogenization will reduce the viscosity of the cream, a result not particularly favored for table cream, but less objectionable than cream producing flakes in coffee.

Under some conditions, additions of milk solids in the form of high quality condensed skimmilk or skimmilk powder will raise the heat coagulation point of homogenized cream. Excessive additions to any cream or any addition to some creams may cause loss of stability, however.

The temperature of homogenization as well as the temperature of preheating is important in preserving the stability of homogenized cream. In general the cream should not be homogenized at a temperature lower than that at which it is preheated unless this is over 170°F. (76.6°C.) for 30 minutes or 180°F. (82.2°C.) flash.

Salt stabilizers used for improving the stability of homogenized cream toward heat should be added prior to homogenization for maximum results, since they not only stabilize by correcting the "salt balance" but also quite as much, perhaps, by hindering the formation of fat clumps during homogenization.

As a general rule, the heat coagulation point of sterile cream and the tendency of homogenized table cream to feather in coffee, parallels the degree of fat clumping but in an inverse fashion.

REFERENCES

(1) Webb, B. H. 1930 The sterilization of sweet cream for market purposes. Jour. Dairy Sci., xiii, no. 3, 159.

(2) Doan, F. J. 1921 Unpublished data obtained at an evaporated milk plant.

(3) Deysher, E. F., Webb, B. H., and Holm, G. E. 1929 The relations of temperature and time of forewarming of milk to the heat stability of its evaporated product. Jour. Dairy Sci., xii, no. 1, 80.

(4) Webb, B. H., and Holm, G. E. 1928 The heat stability and feathering of sweet cream as affected by different homogenization pressures and different temperatures of forewarming. Jour. Dairy Sci., xi, no. 3, 243.

(5) Tracy, P. H., and Ruehe, H. A. 1928 A case of cream feathering. The Creamery and Milk Plant Monthly, xvii, no. 5, 21.

(6) Sommer, H. H., and Hart, E. B. 1926 The heat coagulation of evaporated milk. Wis. Agr. Exp. Sta. Res. Bul. no. 67.

(7) Tracy, P. H., and Ruehe, H. A. 1930 Factors affecting the heat coagulation of homogenized coffee cream. Ill. Agr. Exp. Sta. Bul. 352.

(8) Hening, J. C., and Dahlberg, A. C. 1929 The effects of certain salts on the physical properties of ice cream mixes. Jour. Dairy Sci., xii, no. 2, 129.

(9) Hening, J. C. 1928 The temperature of pasteurization—how it affects the mix properties. Ice Cream Trade Jour., xxiv, no. 10, 53.

(10) Doan, F. J. 1929 Some factors affecting the fat clumping produced in milk and cream mixtures when homogenized. Jour. Dairy Sci., xii, no 3, 211.

(11) Doan, F. J. 1930 Protein stability affected by homogenization of mixes. Ice Cream Review, xiii, no. 8, 40.

(12) Martin, W. H., and Dahle, C. D. 1925 The use of the two-stage valve. 38th Ann. Report, Pa. Agri. Exp. Sta 23.

(13) Doan, F. J. 1927 Viscolized milk and its detection. Jour. Dairy Sci., x, no 6, 501.

PROCEEDINGS OF THE TWENTY-SIXTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The twenty-sixth Annual Meeting of the American Dairy Science Association was held at the College of Agriculture, University of California, Berkeley and Davis, California, July 13 to 15. This is the first time that a meeting of the Association has been held on the Pacific Cost. The attendance was very satisfactory there being approximately 150 representatives of the industry present from all sections of the United States and several provinces of Canada.

A number of members and their families took advantage of the itinerary arranged for those who came by way of Southern California. On July 7 as guests of the Los Angeles Chamber of Commerce they made a tour of moving picture studios and many other points of interest in and around Los Angeles. They were luncheon guests of the Los Angeles Milk Distributors at Casa del Mar Club, Santa Monica.

On July 8 as guests of the Southern California Milk Dealers' Association they visited other points of interest in Los Angeles, Pasadena and the San Fernando Valley. The Challenge Creamery and Butter Association at Los Angeles and the Arden's Certified Dairy at El Monte were visited during the morning and at noon lunch was served by the Adohr Stock Farms, Van Nuys.

On July 9 an auto caravan, which included free transportation furnished by the Dairy Plant Operators of the San Joaquin Valley, made the trip from Merced to the Yosemite Valley. The following morning the party left Yosemite for San Francisco. At noon they were served lunch as guests of the Golden State Company, Ltd. After lunch the milk powder and sweet cream plant of this company at Los Banos was visited.

During the morning of July 11 transportation was furnished by the San Francisco Milk Distributors for a trip to Stanford University and Millbrae Dairy. After inspecting this herd lunch was served on the Mills Estate by the Millbrae Dairy. In the afternoon there was instruction in judging dairy products at the San Francisco plant of the Golden State Company, Ltd.

On Monday morning, July 13, the group ferried across to Oakland and after a tour of the University of California campus settled down to the official program which started at Berkeley and was continued at Davis during the next two days.

During the formal meetings at Davis on July 14 and 15 the ladies were given a special two day trip through the Sierra Nevada Mountains to Lake Tahoe and return.

Those in attendance were enthusiastic in their praise of Dr. Roadhouse and his associates for the completeness of their arrangements for the comfort and entertainment of the visitors.

The following program was presented:

July 13, 1931, University of California Campus, Berkeley

9:30 Tour of University of California Campus, Berkeley—G. E. Gordon in charge.

International House

12:00 Cafeteria Luncheon.

12:45 Address: Influence of International House on University Life—A. C. Blaisdell—Auditorium, International House.

Giannini Hall

1:45 Address of Welcome: C. B. Hutchison, Dean, College of Agriculture.

2:15 Address: The Dairy Extension Program in California—B. H. Crocheron, Director, Agricultural Extension.

University Farm, Davis

8:00 Registration: Auditorium, Class Room Building.

GENERAL SESSION

University Farm, Davis, Tuesday, July 14, 1931—Auditorium, Class Room Building

H. B. **Ellenberger**, President, presiding

8:30 Call to order.

Address of Welcome—W. L. Howard, Director, Branch College of Agriculture, Davis.

8:45 President's Address—H. B. Ellenberger, University of Vermont Announcements.

9:45 The Inorganic Phosphorus Content of the Blood Plasma as an Indication of Phosphorus Deficiency in Cattle—C. H. Eckles, L. S. Palmer, T. W. Gullickson, University of Minnesota.

10:00 Salt Sick—A Naturally Occurring Nutritional Anemia in Cattle—W. M. Neal, University of Florida.

10:15 The Present Dairy Situation—H. R. Tolley, University of California.

10:30 The Application of Science to Marketing Dairy Products—Sam H. Greene, California Dairy Council.

10:45 Calcium and Phosphorus Balances with Milking Cows through Full Lactation Periods—H. B. Ellenberger, J. A. Newlander, C. H. Jones, University of Vermont.

11:00 The Ability of Dairy Cows to Withstand Exposure to Cold Weather—J. R. Dice, North Dakota Agricultural College.

11:15 Some Studies in the Physiology of Fats—H. M. Evans, S. Lepkovsky, University of California.

11:30 Problems of Undulant Fever in the West—Karl F. Meyer, Director, Hooper Foundation Medical Research, San Francisco.

11:45 Some Effects of Variations in Temperature and Humidity on the Dairy Cow—W. M. Regan, G. A. Richardson, University of California.

12:00 Buffet Luncheon—Dairy Industry Building, Room 205.

DAIRY PRODUCTION[1]

Tuesday, 1:30 p.m.—Room 147, Animal Science

P. M. Brandt, Chairman, Dairy Production Section, presiding

1:30 Gestation Period of Holstein Cattle—J. C. Knott, Washington State College.

1:45 Dry Skim Milk Rations vs. Blood Flour Rations for Calf Raising—S. I. Bechdel, Pennsylvania State College.

2:00 Experiments Regarding the Drying Up of Cows—Ralph Wayne, University of Minnesota.

2:15 Experiments Regarding the Use of Rye as a Feed for Dairy Cattle—Henry Hirst, University of Minnesota.

2:30 Mangels versus Silage for Milking Cows—Earl Weaver, A. H. Kuhlman, Oklahoma Agricultural College.

2:45 The Influence of Fat and Curd Tension upon the Digestibility of Milk for Calves—C. Y. Cannon, Iowa State College.

3:00 The Physiological Effect of Rations Restricted Principally or Solely to the Alfalfa Plant—J. R. Haag, Oregon State Agricultural College.

3:15 Proper Recognition for Production Records in our Dairy Shows—C. E. Wylie, University of Tennessee.

3:30 Blood Volume Determination in the Live Dairy Cow and its Significance —H. A. Herman, C. W. Turner, University of Missouri.

3:45 The Influence of the Number of Daily Milkings upon Production of Dairy Cows - R. F. Morgan, H. P. Davis, University of Nebraska.

4:00 A Visit to the Dairy Barn and Inspection of Specialized Apparatus in Animal Science Building.

---

[1] Concurrent with this Program, Roy C. Jones, Bureau of Dairy Industry, U. S. D. A., will give an Herediscope Demonstration especially designed for Dairy Extension Workers—Animal Science, Room 151.

DAIRY MANUFACTURES

Tuesday, 1:30 p.m.—Auditorium, Class Room Building

C. L. ROADHOUSE, Chairman, Dairy Manufactures Section, presiding

1:30 Pasteurization and Cooling of Milk in a Vat to Secure Optimum Creaming —J. C. Marquardt, A. C. Dahlberg, New York Experiment Station.

1:45 A Study of the Quality of Butter Made from Refrigerated Cream Held on Farms for Twice Weekly Shipments—G. Wilster, Oregon State Agricultural College.

2:00 The Heat Coagulation of Milk from Individual Cows during a Lactation Period—B. H. Webb, E. F. Deysher, G. E. Holm, Bureau of Dairy Industry, U. S. D. A.

2:15 Simplified Test for the Detection of Neutralized Cream—J. I. Keith, Oklahoma Agricultural College.

2:30 Protein Decomposition Studies in Cream and Butter—H. A. Bendixen, Washington State College.

2:45 Viability of Lactobacillus Acidophilus as Affected by Freezing in a Sherbert Mixture—C. C. Prouty, Washington State College.

3:00 The Influence of the Various Monosacchride and Diacchride Sugars on the Crystallization Point of Lactose in Ice Cream—J. H. Erb, Ohio State University.

3:15 Manufacture and Tests of High-Quality Domestic Casein—E. O. Whittier, Bureau of Dairy Industry, U. S. D. A.

3:30 Chemical Study of Casein Made by Natural Sour Method in Eight Idaho Plants—D. R. Theophilus, H. C. Hansen, R. S. Snyder, University of Idaho.

3:45 Comparative Efficiency of Cream Collection by Cream Stations, Truck Routes and Direct Shipping—C. O. Youngstrom, D. R. Theophilus, F. W. Atkeson, University of Idaho.

4:00 A Visit to the Dairy Industry Building.

SYMPOSIUM ON EDUCATION

Tuesday, 8:00 p.m.—Auditorium, Class Room Building

H. B. ELLENBERGER, presiding

8:00 The Psychology of Learning—G. M. Ruch, University of California.

Open Discussion and Questions.

Curricula for Dairy Students.

- (a) The Curriculum for Dairy Production—P. M. Brandt, Oregon State Agricultural College.
  Discussion: W. M. Regan, University of California.
- (b) The Curriculum for Dairy Manufactures—H. W. Nisonger, Junior Dean, Ohio State University.
  Discussion: R. B. Stoltz, Ohio State University.

Organization of Instruction in Dairy Machinery—L. C. Thomsen, University of Wisconsin.

Discussion: C. L. Roadhouse, University of California.

DAIRY PRODUCTION

Wednesday, July 15, 8:30 a.m.—Room 147, Animal Science

P. M. Brandt, Chairman, Dairy Production Section, presiding

8:30 Water Requirements of Dairy Calves—F. W. Atkeson, G. C. Anderson, T. R. Warren, University of Idaho.

8:45 The Inheritance of Hernia in Holstein Cattle—T. R. Warren, F. W. Atkeson, University of Idaho.

9:00 Irradiation of the Cow's Udder with a Quartz Mercury Lamp as a Treatment for Mastitis—H. C. Hansen, V. A. Cherrington, E. M. Gildow, F. W. Atkeson, University of Idaho.

9:15 A Mathematical Expression of the Relation of Fleshing to Skeleton and its Application to Beef and Dairy Production Researches—P. W. Gregory, H. R. Guilbert, S. W. Mead, University of California.

9:30 Pasture Studies in California—A. Folger, University of California.

9:45 Some Investigations in the Fibre Requirements of Ruminants—S. W. Mead, Harold Goss, University of California.

10:00 Measuring the Returns from Pasture with Dairy Cattle—P. M. Brandt, Oregon State Agricultural College.

10:15 Sterility and Difficult Breeding in Dairy Cattle—I. R. Jones, Oregon State Agricultural College.

10:30 The Effect of Various Mineral Supplements on Calcium Phosphorus and Nitrogen Metabolism of Dairy Cattle—J. R. Haag, Oregon State Agricultural College.

10:45 The Use of the Ovarian Hormone for Diagnosing Pregnancy in Dairy Cattle—C. W. Nibler, University of Nebraska.

11:00 Vitamin A and D Requirements of Dairy Calves—I. W. Rupel, G. Bohstedt, E. B. Hart, University of Wisconsin.

11:15 Section Meeting, Committee Reports, Business.

DAIRY PRODUCTS AND BACTERIOLOGY

Wednesday, 8,30 a.m.—Auditorium, class room building

C. L. Roadhouse, Chairman, Dairy Manufactures Section, presiding

8:30 An Alkaline Reagent as a Substitute for Acid in Testing Dairy Products by the Babcock Method—W. E. Petersen, University of Minnesota.

8:45 The Effect of Flash Pasteurization on the Creaming of Milk—A. C. Dahlberg, J. C. Marquardt, New York Experiment Station.

9:00 The Effect of Various Salts upon the Heat Coagulation of Milks of Different Concentrations—B. H. Webb, G. E. Holm, Bureau of Dairy Industry, U. S. D. A.

9:15 Tests of Ice Refrigerted Cooling Units under Control Conditions—R. L. Perry, University of California.

9:30 A study of Methods for Determining Numbers of Molds and Yeasts in Butter—E. G. Hood, A. H. White, Department of Agriculture, Ottawa, Canada.

9:45 Factors Influencing the Freezing Properties, Stability and Physical Properties of Chocolate Ice Cream—W. H. E. Reid, W. E. Painter, Univeristy of Missouri.

10:00 Checking the Age of Ice Cream as a Basis of Quality Control—H. F. Judkins, National Dairy Products Corporation, New York.

10:15 The Mechanical Effect upon Heat Coagulation of Fat Dispersed by Homogenization—B. H. Webb, G. E. Holm, Bureau of Dairy Industry, U. S. D. A.

10:30 A Study of the Physical and Chemical Properties of Cottage Cheese Manufactured from Skim Milk Powder—W. H. E. Reid, C. L. Fleshman, University of Missouri.

10:45 A Study in the Manufacture of Gorgonzola Type Cheese—C. A. Phillips, Fred Kopp, University of California.

11:00 Section Meeting, Committee Reports, Business.

EXTENSION SECTION

Wednesday, 8 30 a.m --Room 151, Animal Science

M. J. Regan, Chairman, Dairy Extension Section, presiding

8:30 How Far Can We Go in Recommending a Dairy Ration with a Minimum Grain Mixture—George B. Caine, Utah State Agricultural College.

8:45 Methods of Handling Dairy Herd Improvement Association Work in Central States—J W. Linn, Kansas State College.

9.00 Enterprise Efficiency Studies on Dairy Farms—A. Shultis, L. W. Fluharty, University of California

9:15 Does High Production Pay?—J. C. McDowell, Bureau of Dairy Industry, U. S. D. A.

9:30 Recent Development in Official Testing—H. N. Colman, Oregon State Agricultural College.

9:45 Quality Dairy Products—M. L. Flack, University of Nebraska.

10:00 Results of Purebred Sire Contest as Conducted in Iowa—Floyd Johnston, Iowa State College.

10:15 Breed Extension Projects as Part of the Dairy Extension Program—D. L. Fourt, University of Idaho.

10:30 Theoretical Advantages of Cooperative Bull Associations Compared with Actual Results Obtained—F. W. Atkeson, University of Idaho.

10:45 Calculating Dairy Rations—F. B. Headley, University of Nevada.

11:00 Business Meeting of Section.

GENERAL PROGRAM

Wednesday, 1:30 p.m.—Auditorium, Class Room Building

H. B. Ellenberger, presiding

1:30 Calcium and Phosphorus Metabolism during Pregnancy and Lactation on a Low Calcium Diet—Harold Goss, University of California.

1:45 Some Effects of Low Protein Diets on the Estrus Cycle and Reproduction in the Rat—Harold Guilbert, Harold Goss, University of California.

2:00 Activation of Impotent Males with an Ovary Stimulating Hormone—H. H. Cole, Harold Guilbert, Harold Goss, University of California.

2:15 The Effect of Feeding Concentrated Coloring Substances to Dairy Cows on the Color of Milk and Milk Fat—W. D. Swope, Pennsylvania State College.

2:30 A New Technique for Obtaining Standard Shades in Butter—E. G. Hood, A. H. White, Department of Agriculture, Ottawa, Canada.

2:45 The Source of Thermophilic Bacteria—C. S. Mudge, J. L. Henderson, University of California.

3:00 The Effect of Some Feeds on Certain Properties of Milk—G. A. Richardson, University of California.

3:15 State Grading of Milk for Dairy Manufacture by Direct Count—M. E. McDonald, State Department of Agriculture, Sacramento.

3:30 Studies in Increasing Antirachitic Potency of Cow's Milk—B. H. Thomas, Iowa State College; Florence L. MacLeod, Columbia University.

3:45 The Present Status of the Milk Sugar Problem—E. O. Whittier, Bureau of Dairy Industry, U. S. D. A.

4:00 Business Meetings of Sections.

7:00 Banquet—California Inn, University Farm Campus.

Secretary Graves reported by letter that each of the four proposals to amend the Constitution were carried by large majorities as the result of balloting by mail during the winter. As a result Article 6 now reads as follows:

ARTICLE 6. The officers of this Association shall be President, Vice-President, Secretary-Treasurer, and Journal Editor. The President and Vice-President shall be elected by vote of the general membership and the term of office shall be one year beginning January first. The Secretary-Treasurer and the Journal Editor shall be appointed by the Executive Committee and the term of office shall be at the discretion of the Committee.

"The Preisdent, Vice-President and the chairmen of the sections shall constitute the Executive Committee. In case of the absence of a chairman of a section the next ranking officer is authorized to represent the section at any meeting of the Executive Committee."

The Executive Committee met on both July 13 and 14. Those present were Ellenberger, Wylie, Brandt, Roadhouse and Gordon. There was no one present to represent the Dairy Economics section.

Action taken by the Executive Committee included the following:

The Official Testing section is to be discontinued in accordance with the decision of that section at the Ames meeting last year and the Breeds Relation Committee is to function as a committee of the Production section.

It was voted to be the sense of the Executive Committee that the chairman and seretary of each section and division shall submit to the general secretary minutes, list of officers, and list of committees with their duties and that the general secretary shall furnish copies of this information to the new officers of the Association, its sections and divisions, and that the general secretary shall be responsible for obtaining and distributing this information.

The President was authorized to appoint a committee to act in an advisory capacity with the "Century Dairy Exhibit, Inc." of the Century of Progress Exposition to be held in Chicago.

It was voted that the Constitution of the Association as amended be published in the Journal of Dairy Science with 100 reprints for distribution to new officers as elected.

Mr. R. R. Graves was appointed to continue as Secretary-Treasurer with a salary of $200 per year.

Dr. A. C. Dahlberg was appointed to continue as Journal Editor. Because of increased responsibilities in connection with the program explained hereafter his salary was increased to $500 per year beginning July 1, 1931.

Dr. A. C. Dahlberg presented an extensive report of the committee appointed to investigate future publishing contracts for the Journal of Dairy Science. This report was also presented to the general business meeting which meeting voted to recommend it to the Executive Committee for approval. The Executive Committee voted to approve this report for a new contract for the Journal of Dairy Science subject to the final approval of the Journal Management Committee to be appointed by the President. President Ellenberger has appointed the following as members of the Journal Management Committee:

O. F. Hunziker
R. R. Graves
A. A. Borland

The President has appointed F. W. Bouska to serve in an advisory capacity with the "Century Dairy Exhibit, Inc."

In the general business session the Executive Committee was authorized to incorporate when and in such manner as may seem most desirable to the welfare of the Association and the publication of the Journal of Dairy Science.

The report of the Committee on Bacteriological Methods of Analyzing Dairy Products was received and filed.

The report of the National Dairy Improvement Committee was read and it was voted to continue this committee for another year. The members will be appointed by the President in the near future.

Professor Ragsdale of Missouri announced that arrangements had been made for headquarters at the Missouri Hotel during the National Dairy Show at St. Louis and that the banquet at which the results of the Students Judging Contest are to be announced will be held in the same hotel.

The Committee on Dairy Products Judging was authorized to arrange for a banquet at the time of the National Dairy Exposition for announcement of results of the judging contest.

A Nominating Committee consisting of P. M. Brandt, A. C. Ragsdale, R. W. Bell, W. D. Swope and J. R. Dice submitted the following report:

For President:
- C. E. Wylie, Tennessee
- E. L. Anthony, Michigan

For Vice-President:
- R. B. Stoltz, Ohio
- W. V. Price, Wisconsin

The following resolutions were presented and adopted:

"WHEREAS, our friend and fellow-worker, J. H. McClain, a pioneer in dairy extension work in this country, has been called from his earthly labors after having done so much toward the development and improvement of dairying in all parts of the United States, but more especially in his beloved Southland, WE, the members of the American Dairy Science Association, assembled in Davis, California, July 15, 1931, take this opportunity to express our sympathy to his beloved wife and son. A copy of this resolution is to be sent immediately by our Secretary to Mrs. J. H. McClain of Campebell, South Carolina.

"WHEREAS, Dr. Stephen Moulton Babcock, whose scientific discoveries and investigations have made him justly famous throughout the world, has recently been called from his earthly labors, WE, the members of the American Dairy

Science Association now assembled in Davis, California, desire to express our words of sympathy to his co-workers in the University of Wisconsin, and especially to his brother and other relatives. In losing Dr. Babcock, the world has lost a great and good man and our Dairy Industry has lost one of its greatest benefactors. While time shall last and man shall strive for improvement, the influence of our faithful friend and eminent leader shall continue and the world shall always be a better place in which to dwell because for 87 short years he lived among us.

"WHEREAS, the American Dairy Science Association through invitation of its Western Division has held its annual meeting on the Pacific Coast for the first time, and

"WHEREAS, this Western Division in cooperation with several of the Dairy Departments represented in its territory and with several other dairy organizations, particularly the California Dairy Council, have arranged so excellently for our entertainment and program

"*Be It Resolved* that we express our appreciation to all those who have planned so carefully for our convention and especially to Dr. C. L. Roadhouse and his co-workers and to our Western Division.

Respectfully submitted,
R. B. STOLTZ,
J. C. MCDOWELL,
F. W. ATKESON,
C. Y. CANNON,
A. C. DAHLBERG."

In the Production section it was pointed out that apparently the intent of the uniform herd test rules was not understood thoroughly and that some states were not following the uniform rules in conducting the test, certain ones apparently regarding the herd test as official while others regard it as an association record. It was stated that it was the intent of the breed associations to regard the herd test as official. It was proposed that the necessity of clarifying the situation should be called to the attention of the Breeds Relation Committee.

Chairman Brandt appointed the following committees:

Committee on Evaluation of Proven Sires

H. O. Henderson, *Chairman*
F. W. Atkeson
C. W. Turner
R. R. Graves
J. L. Lush

Committee on the Standardization of Production Records

W. M. Regan, *Chairman*
M. H. Frohman
W. E. Petersen
H. P. Davis
I. R. Jones

Committee on Students' Dairy Cattle Judging Contests

J. E. Dorman, *Chairman*
Warren Gifford
I. W. Rupel
Earl Weaver
C. E. Wylie

Committee on Breeds Relations

J. B. Fitch, *Chairman*
E. L. Anthony
R. T. Harris
G. C. White
E. V. Ellington

G. Bohstedt was elected Chairman and T. W. Gullickson Secretary for the coming year.

In the Dairy Manufacturing section progress reports were submitted from the sub committees on Chemical Methods of Testing Dairy Products. These dealt with "Testing Milk and Cream for Fat" and "Testing Ice Cream for Fat."

The Committee on Judging Dairy Products was empowered to make changes in the rules governing the Students' National Dairy Products Judging Contest.

The following committee appointed by Chairman Roadhouse is to consider the question of Dairy Engineering curricula and report at next year's meeting:

H. A. Bendixen, *Chairman*
R. L. Perry
M. P. Mortensen

R. B. Stoltz was elected Chairman and E. G. Hood Secretary for the coming year.

In the Extension section M. J. Regan was elected Chairman, M. L. Flack, Vice Chairman and C. R. Gearhart Secretary for the coming year.

On July 16 the California Dairy Council provided transportation for all who desired same for a trip to Hartsook Lodge on the Redwood Highway by Williams and Lake County. The Lake County Farm Bureau provided a barbecue lunch at Buckingham Point. In the evening a program was provided by the California Dairy Council.

INDEX TO VOLUME XIV

ACID caseins, Properties of some 337
(Acidity) A study of methods for determining numbers of molds and yeasts in butter. 1. The relation of the pH of the medium 463
Acidity of cows' milk, The effect of dilution on the titratable 136
Acidity on lactobacillus acidophilus cultures, The effect of 198
Acidophilus and lactobacillus bulbaricus cultures stored at various temperatures, Viability of lactobacillus 59
Acidophilus cultures, The effect of acidity on lactobacillus 190
Agglutinin, complement, and chemical composition of the colostrum of the cow 156
Alfalfa ash, The effect of the addition of cod liver oil and 283
American Dairy Science Association, Proceedings of the 26th annual meeting of the 540

BACTERIA) Micrococcus cause of malt flavor in raw milk 457
(Bacteria) Observations on the heat resistance of some ropy milk organisms 27
(Bacteria) The germicidal action of raw milk upon an organism of the sweet curdling type 276
(Bacteria) Variations in the coagulation and proteolysis of milk by streptococcus lactis 40
Bacterial counts presented by Brew and Dotterrer and analyzed by Brew, Further remarks on data on 477
Baker, G. A. Further remarks on the data on bacterial counts presented by Brew and Dotterrer and analyzed by Brew. 477
Bell, R. W., and Gould, S. P Properties of some acid caseins. 337
Black, L. A., and Harris, J. C. The effect of acidity on lactobacillus acidophilus cultures 198
Black, Luther A. Viability of lactobacillus acidophilus and lactobacillus bulgaricus cultures stored at various temperatures 59
Blood of cows and bulls, The hemoglobin and total phosphorus in the 373
Bromcresol green paper in the manufacture of grain-curd casein 26
Brooks, H. J. The influence of environmental temperature on the percentage of butterfat in cows' milk 483
Brown, J. B., and Sutton, T. S. The effect of feeding Menhaden (fish) oil on the secretion of milk and the composition of butter fat in the dairy cow 125
Buffer value of herd milk, Variations in the. 50
Bulls, The hemoglobin and total phosphorus in the blood of cows and 373
Butter. 2, A study of methods for determining numbers of molds and yeasts in 494
Butter, Method for hydrogen ion determination of 347
Butter, The churn as a source of molds in 398

Butterfat in cows' milk, The influence of environmental temperature on the percentage of 483

Butterfat in the dairy cow, The effect of feeding Menhaden (fish) oil on the secretion of milk and the composition of...... 125

Butterfat production and annual feed cost of dairy cows under farm conditions, Correlation between annual.... 73

(Butterfat production) Uniform rules for the herd test...... . 394

Butterfat, The contribution of the dam in inheritance of milk and. 379

Butterfat to ultra violet radiations, Fat soluble vitamins. XXXII. The determination of vitamin A and its stability in... 229

CALCIUM and phosphorus metabolism in dairy cows. IV. The assimilation of calcium fed as calcium gluconate 268

Calcium assimilation as indicated by bone analysis in long time experiments. 322

Calcium assimilation. XIV. The influence of mineral acids and sugar on the calcium metabolism of milking cows, Dietary factors influencing...... 307

Calves, Deficiencies in rations devoid of roughage for 283

Calving interval and average milk yield, Length of... 294

Casein, Bromcresol green paper in the manufacture of grain-curd 26

Caseins, Properties of some acid . 337

Cattle, An interpretation of the feeding standards for growing dairy..... 116

Churn as a source of molds in butter, The.... 398

Churn, sanitation, Preliminary studies on...... 404

(Coagulation) A test for the detection of milk unstable to heat... 93

Coagulation and proteolysis of milk by streptococcus lactis, Variations in the 40

Coagulation by heat and rennet and upon separation of the fat, Effect of single and double homogenization of cream upon.. . 508

Cod liver oil and alfalfa ash, Deficiencies in rations devoid of roughage for calves. I. The effect of the addition of.... 283

Colostrum of the cow, Agglutinin, complement, and chemical composition of the... 156

COMBS, W. B. (See Morrison, H. B.). . . 404

COMBS, W. B. (See Macy, H.) . 398

Complement, and chemical composition of the colostrum of the cow, Agglutinin 156

Composition of the colostrum of the cow, Agglutinin, complement, and chemical 156

COPELAND, LYNN. The contribution of the dam in inheritance of milk and butterfat. 379

Copper under various conditions, The solubility of metals in milk. 1. The solubility of 416

CORDES, W. A. (See Hunziker, O. F.).............. 347

Cows and bulls, The hemoglobin and total phosphorus in the blood of 373

Cow and efficiency of milk production, Size of..... 14

Cow, The effect of feeding Menhaden (fish) oil in the secretion of milk and the composition of butter fat in the dairy......... 125

Cows, Calcium and phosphorus metabolism in dairy cows. IV. The assimilation of calcium fed as calcium gluconate 268

Cows, Calculating rations for dairy 173

Cows, Dietary factors influencing calcium assimilation. XIV. The influence of mineral acids and

sugar on the calcium metabolism of milking...... 307
Cows, Skimmilk as a feed for dairy. 189
Cows under farm conditions, Correlation between annual butter fat production and annual feed cost of dairy. . . . 73
Cream of the development of swell in ice cream, The influence of sweetened, frozen . . . 221
Cream, The effect of the hydrogen ion concentration on the churning time of 1
Cream to fat clumping produced by homogenization, The relation of feathering and heat stability of 528
Cream upon coagulation by heat and rennet and upon separation of the fat, Effect of single and double homogenization of 508
CURRAN, HAROLD, R. The germicidal action of raw milk upon an organism of the sweet curdling type. 276

DAM in inheritance of milk and butterfat, The contribution of the 379
Daughters necessary to prove a sire, The number of . . 209
Dietary factors influencing calcium assimilation. XIV. The influence of mineral acids and sugar on the calcium metabolism of milking cows 307
DOAN, F. J. The relation of feathering and heat stability of cream to fat clumping produced by homogenization . . . 527
Dow, O. D. (See Supplee, G. C.). 447

ECKLES, C. H., and SHULTZ, E. N. Skimmilk as a feed for dairy cows 189
EBANS, FRED R. (See Ramsdell, Guy A.).................... . 93
Evaporated milk in hot coffee, The feathering of 177

FAT clumping produced by homogenization, The relation of feathering and heat stability of cream to 527
Fat, Effect of single and double homogenization of cream upon coagulation by heat and rennet and upon separation of the . 508
Fat soluble vitamins. XXXIII. The determination of vitamin A and its stability in butter fat to ultra violet radiations 229
Feathering and heat stability of cream to fat clumping produced by homogenization, The relation of 527
Feathering of evaporated milk in hot coffee, The . 177
Feed cost of dairy cows under farm conditions, Correlation between annual butter fat production and annual . . 73
Feed for dairy cows, Skimmilk as a 189
Feeding Menhaden (fish) oil on the secretion of milk and the composition of butter fat in the dairy cow, The effect of . .. 125
Feeding Standards for growing dairy cattle, An interpretation of the 116
FITCH, J. B., and ANTHONY, E. L. Uniform rules for the herd test . 394
FITCH, J. B., and LUSH, R. H. An interpretation of the feeding standards for growing dairy cattle 116
FLANIGAN, G. E. (See Supplee, G. C.) . . . 447
Flavor development in market milk, The relation of certain plant processes to 250
Flavor in raw milk, Micrococcus cause of malt... 457
Frozen cream on the development of swell in ice cream, The influence of sweetened 221

GAINES, W. L., and PALFREY, J. R. Length of calving interval and average milk yield...... 294

GAINES, W. L. Size of cow and efficiency of milk production.... 14

GEBHARDT, H. T., and SOMMER, H. H. The solubility of metals in milk I. The solubility of copper under various conditions....... 416

Germicidal action of raw milk upon an organism of the sweet curdling type, The.................... 276

GOULD, S. P. (See Bell, R. W.).. 337

GUTHRIE, E. S., and SHARP, PAUL FRANCIS. The effect of hydrogen ion concentration on the churning time of cream .. 1

HALE, WALTER S. (See Turner, William A.)........ ... 268

HAMMER, B. W., and HUSSONG, R. V. Observations on the heat resistance of some ropy milk organisms.... .. 27

HAMMER, B. W. (See Harriman, L. A.... 40

HARRIMAN, L. A., and HAMMER, B. W. Variations in the coagulation and proteolysis of milk by streptococcus lactis.......... 40

HARRIS, J. C. (See Black, Luther A.).............................. 198

HARTMAN, ARTHUR M., and MEIGS, EDWARD, B. Calcium assimilation as indicated by bone analysis in long-time experiments .. 322

Heat, A test for the detection of milk unstable to 93

Heat resistance of some ropy milk organisms, Observations on the.. 27

Heat stability of cream to fat clumping produced by hemogenization, The relation of feathering and. 527

Hemoglobin and total phosphorus in the blood of cows and bulls, The........................... 373

HENING, J. C. Some observations on the basic viscosity of ice cream mixes....... 84

Herd test, Uniform rules for the.. 394

Homogenization of cream upon coagulation by heat and rennet and upon separation of the fat, Effect of single and double..... 508

Homogenization, The relation of feathering and heat stability of cream to fat clumping produced by 527

HOOD, E. G. (See White, A. H.). 463

HOOD, E. G. (See White, A. H.). 494

HUNZIKER, O. F., CORDES, W. A., and NISSEN, B. H. Method for hydrogen ion determination of butter.... 347

HUSSONG, R. V. (See Hammer, B. W.)..... . .. 27

(Hydrogen ion concentration) Bromcresol green paper in the manufacture of grain-curd casein. 26

Hydrogen ion concentration of the churning time of cream, The effect of the 1

Hydrogen ion determination of butter, Method for 347

ICE cream and sherbet, Speed of dasher and scraper as affecting the quality of........ 107

Ice cream, The influence of sweetened, frozen cream on the development of swell in..... 221

IKEJAMA, H. (See Sato, M.).... 156

Inheritance of milk and butterfat, The contribution of the dam in............................ 379

JOHNSON, WM. T., JR. (See Ramsdell, Guy A.)............ 93

KANE, EDWARD A. (See Turner, William A.)....... 268

KOHLENBERG, O. J. (See Supplee, G. C.).......................... 447

LACTOBACILLUS acidophilus and lactobacillus bulgaricus cultures stored at various temperatures, Viability of... ... 59
Lactobacillus acidophilus cultures, The effect of acidity on 198
Lactobacillus bulgaricus cultures stored at various temperatures, Viability of lactobacillus acidophilus and 59
Lush, R. H. (See Fitch, J. B.) . 116
Lush, Jay L. The number of daughters necessary to prove a sire 209

Macy, H., Combs, W. B., and Morrison, H. B., Jr. The churn as a source of molds in butter . . . 398
Macy, H (See Morrison, H. B.). 404
McCay, C. M. The hemoglobin and total phosphorus in the blood of cows and bulls . 373
McIntyre, C. W., Correlation between annual butter fat production and annual feed cost of dairy cows under farm conditions . . 73
Mead, S. W., and Regan, W. M. Deficiencies in rations devoid of roughage for calves I. The effect of the addition of cod liver oil and alfalfa ash . . . 283
Meigs, Edward, B. (See Hartman, Arthur M.).. .. . 322
Menos, J. (See Sommer, H. H.) 136
Metabolism in dairy cows. IV. The assimilation of calcium feed as calcium gluconate, Calcium and phosphorus 268
Metabolism of milking cows, Dietary factors influencing calcium assimilation. XIV. The influence of mineral acids and sugar on the calcium................ 307
Metals in milk. I. The solubility of copper under various conditions, The solubility of... 416
Micrococcus cause of malt flavor in raw milk.... 457
Milk and butter fat, The contribution of the dam in the inheritance of 379
Milk by streptococcus lactis, Variations in the coagulation and proteolysis of 40
Milk in hot coffee, The feathering of evaporated..... . . . 177
Milk, Microccocus cause of malt flavor in raw 457
Milk organisms, Observations on the heat resistance of some ropy. 27
Milk production, Size of cow and efficiency of 14
Milk, The effect of dilution on the titratable acidity of cows'.. 136
Milk, The influence of environmental temperature on the percentage of butterfat in cow's.. . 483
Milk, The relation of certain plant processes to flavor development in market . 250
Milk, unstable to heat, A test for the detection of. 93
Milk upon an organism of the sweet curdling type, The germicidal action of raw.... . 276
Milk, Variations in the buffer value of herd . . . 50
Milk yield, Length of calving interval and average . . 294
Molds and yeasts in butter. I. The relation of the pH of the medium, A study of methods for determining number of 463
Molds and yeasts in butter. 2. The influence of temperature and time of incubation, A study of methods for determining numbers of........ 494
Molds in butter, The churn as a source of.... 398
(Molds) Preliminary studies on churn sanitation.. 404

Morrison, H. B., Jr., Macy, H., and Combs, W. B. Preliminary studies on churn sanitation.... 404

Morrison, H. B., Jr., (See Macy, H.) 398

NISSEN, B. H. (See Hunziker, O. F.) 347

OFFICIAL testing) Uniform rules for the herd test... . .. 394

Ogura, K. (See Sato, M.)........ 156

PALFREY, J. R. (See Gaines, W. L.)..... 294

Phosphorus in the blood of cows and bulls, The hemoglobin and total.......... 373

Price, Walter V. The influence of sweetened, frozen cream on the development of swell in ice cream. 221

Proceedings of the 26th annual meeting of the American Dairy Science Association 540

Proteolysis of milk by streptococcus lactis, Variations in the coagulation and .. 40

RAMSDELL, Guy A., Johnson, Wm. T., Jr., and Evans, Fred R. A test for the detection of milk unstable to heat.... . .. 93

Ramsey, R. J. (See Tracy, P. H.). 457

Rations devoid of roughage for calves. I. The effect of the addition of cod liver oil and alfalfa ash, Dcficiencies in...... 283

Rations for dairy cows, Calculating. 173

Regan, W. M. (See Mead, S. W.). 283

Reichart, E. L. Speed of dasher and scraper as affecting the quality of ice cream and sherbet. 107

Ropy milk organisms, Observations on the heat resistance of some.......... 27

Roughage for calves. I. The effect of the addition of cod liver oil and alfalfa ash, Deficiencies in rations devoid of.... 283

Ruehe, H. A. (See Tracy, P. H.). 250

SATO, M., Ogura, K., and Ikeyima, H. Agglutinin, complement, and chemical composition of the colostrum of the cow..... 156

Secretion of milk and the composition of butter fat in the dairy cow, The effect of feeding Menhaden (fish) oil on the 125

Separation of the fat, Effect of single and double homogenization of cream upon coagulation by heat and rennet and upon . 508

Sharp, Paul Francis. (See Guthrie, E. S.) 1

Sherbet, Speed of dasher and scraper as affecting the quality of ice cream and...... .. . 107

Shultz, E. N. (See Eckles, C. H.)....... 189

Sire, The number of daughters necessary to prove a 209

Skimmilk as a feed for dairy cows 189

Sommer, H. H., and Menos, Jules. The effect of dilution on the titratable acidity of cows' milk. .. 136

Sommer, H. H. (See Gebhardt, H. T.)....... .. .. .416

Steenbock, H., and Wirick, Alice M. Fat Soluble vitamins. XXXIII. The determination of vitamin A and its stability in butter fat to ultra violet radiations........ 229

Streptococcus lactis, Variations in the coagulation and proteolysis of milk by........ 40

Supplee, G. C., Kohlenberg, O. J., Flanigan, G. E., and Dow, O. D. The relative antineuritic (vitamin B) potency of certain milk derivatives............... 447

Sutton, T. S. (See Brown, J. B.). 125

TEMPERATURE on the percentage of butterfat in cows' milk, The influence of environmental . . . 483
Test, Uniform rules for the herd 394
TRACY, P. H., and RAMSEY, R. J. Micrococcus cause of malt flavor in raw milk . . . 457
TRACY, P. H., and RUEHE, H. A. The relation of certain plant processes to flavor development in market milk 250
TURNER, WILLIAM A., KANE, EDWARD A., HALE, WALTER S., and WISEMAN, HERBERT C. Calcium and phosphorus metabolism in dairy cows. IV The assimilation of calcium fed as calcium gluconate . . 269

ULTRA Violet radiations, Fat soluble vitamins. XXXIII. The determination of vitamin A and its stability in butter fat to. 229

VISCOSITY of ice cream mixes, Some observations on the basic 84
Vitamin A and its stability in butter fat to ultra violet radiations, Fat soluble vitamins XXXIII. The determination of 229
(Vitamin B) potency of certain milk derivatives, The relative antineuritic. 447

WATSON, PAUL D. Variations in the buffer value of herd milk 50
WEBB, B. H. Effect of single and double homogenization of cream upon coagulation by heat and rennet and upon separation of the fat 508
WHITAKER, RANDALL. The feathering of evaporated milk in hot coffee..... . . 177
WHITE, A. H., and HOOD, E. G. A study of methods for determining numbers of molds and yeasts in butter. I. The relation of the pH of the medium..... 463
WHITE, A. H., and HOOD, E. G A study of methods for determining numbers of molds and yeasts in butter. 2. The influence of temperature and time of incubation. 494
WIRICK, ALICE M. (See Steenbock, H.) 229
WISEMAN, G. (See Turner, William A.) . . . 268
WOODWARD, T. E Calculating rations for dairy cows 173

YEASTS in butter. I. The relation of the pH of the medium, A study of methods for determining numbers of molds and. 463
Yeasts in butter. 2. The influence of temperature and time of incubation. A study of methods for determining numbers of molds and 494

I A R.I. 75

INDIAN AGRICULTURAL RESEARCH
INSTITUTE LIBRARY, NEW DELHI.

Date of Issue	Date of Issue	Date of Issue

GIPNLK—H-40 I.A.R.I.—29-4-55—15,000